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PEOPLE AND EVENTS

News from the Universities

Dr. Bearden New Head of Mississippi Dairy Department

New head of the Dairy Department at Mississippi State University is H. J. BEARDEN, who has returned to his home state from Cornell University where he was an associate professor.

H. J. Bearden

Dr. Bearden was born in Starkville, Mississippi, on May 12, 1926. With his family, he moved to a farm in Walthall County when he was seven years old. He graduated from Enon High School in 1944, spent the next two years in the Navy, and then entered Mississippi State College. While a student he worked at the dairy barn and helped with artificial insemination research. Majoring in Dairy Production, he graduated with special honors in January, 1950.

After earning the Master of Science degree from the University of Tennessee in 1951, Dr. Bearden was awarded the Ralston Purina fellowship for work toward a Ph.D. He earned this degree in 1954 from Cornell University, with a major in animal breeding and minors in animal nutrition and veterinary physiology. In 1954, he joined the Cornell staff as an assistant professor and was promoted to associate professor in 1960. He became head of the dairy department at Mississippi State University and the Mississippi Agricultural Experiment Station October 1, 1960, suceeding J. T. MILES, who returned to the University of Tennessee to become head of its dairy department.

Arizona

Dairying in the Decade Ahead will be the theme of the Annual Dairymen's Conference of April 5, 1961, at Arizona State University, Tempe. According to E. G. MOODY, discussions will center around the political climate, managing the milk supply, and bargaining. Participants will include H. W. HALVORSON, Agricultural Economist, University of Wisconsin; GLENN LAKE, president, National Milk Producers Federation and Michigan Milk Producers Association; RAY SELTZER, Agricultural Economist, University of Arizona; and R. G. LYTLE, manager, United Dairymen of Arizona.

Department Head Appointed at the University of Tennessee

J. T. MILES has been appointed head of the Dairy Department of the University of Tennessee, effective September 1. He will succeed C. E. WYLIE, who is retiring August 31.

J. T. Miles

Dr. Miles is from Erin, Tennessee. He earned the Bachelor of Science degree in 1940, majoring in dairying, and the Master's degree at the University of Tennessee in 1948. In 1951, he received his Doctor of Philosophy degree at the University of Wisconsin. Major studies have been in dairy and minors in Agronomy and Biochemistry. He is now head of the Dairy Department at Mississippi State University.

In addition to experience at Mississippi State, Dr. Miles was herdsman and assistant superintendent at the Dairy Experiment Station, Lewisburg, from 194[illegible] to 1941. He was associate professor, then professor on the Mississippi State staff between July, 1951, and August, 1960, except for 1953-54, when he was on the Iowa State University staff as assistant professor.

Dr. Miles is a member of the American Dairy Science Association and the American Society of Animal Production. Fraternal memberships include Phi Kappa Phi and Sigma Xi. He is author or co-author of over 35 scientific articles or publications in his area of specialization.

California

Look-Ahead Conference

B. E. HUBBELL was program chairman for the annual California Dairy Industry Conference held at the University of California, Davis, Jan. 30, 31, Feb. 1, 1961. About 400 people registered for the three-day event. Among the prominent speakers on the program were H. A. BENDIXEN, Washington State University, R. F. HOLLAND, Cornell, R. J. WERNER, former executive secretary, Milk Industry Foundation, and G. M. BRIGGS, Department of Nutrition, University of California at Berkeley.

The theme of the Conference was A Look Ahead, and 42 papers relating to this theme were presented in the various sessions.

At the annual banquet, STERLING LAMPERT, McColl's Dairy, Redding, was installed as president of the California Dairy Industries Association, co-sponsors with the University

of the Conference. J. B. CLOSE, Borden Company, Sacramento, won the Grand Prize for product judging.

J. B. MICKLE of Oklahoma State University is spending a six-month's sabbatical leave in the Department of Food Science and Technology at the University of California at Davis. While there Dr. Mickle will carry on post-doctoral studies on the chemistry of milk fat in collaboration with E. L. JACK and L. M. SMITH.

Connecticut
Animal Breeding Short Course

People from seven states braved severe winter weather and were enrolled in an intensive one-week short course in Animal Breeding at the University of Connecticut for the week of January 30 through February 3. Sessions ran well into the evenings and discussion periods and demonstrations supplemented the lecture material. One of the most popular laboratory periods was the anatomy and function of twenty bull and cow reproductive tracts in different stages of pregnancy or abnormality. The herds at the University were used for demonstration and teaching purposes. Those attending expressed high interest in the program and most faced the immediate task of better analyzing their own herd when they returned home.

The physiology of reproduction, causes of low reproductive efficiency, fundamentals of genetics, systems of breeding, and practical selection and performance testing were stressed. The group included those interested in beef cattle, dairy cattle, and horse breeding. Joint sessions of the entire group were conducted approximately half of the time. The remainder of the time was spent in separate sections for the separate interest groups.

The objective of the course was to serve adults working with the various livestock species and better inform them of the principles of animal reproduction, inheritance, and selection. There was no charge for this course.

It was sponsored by the Animal Industries Department of the College of Agriculture in cooperation with the Department of Animal Diseases. Eleven members of both departments were involved with the program. A similar course is tentatively planned for 1962.

The next short course at the University of Connecticut along the lines of Animal Breeding is the one scheduled for March 27-31, on Artificial Insemination. It is a practical course for cattlemen who want to master the techniques of semen collection, evaluation, and processing and insemination techniques. Further information on it can be obtained by contacting A. I. Mann, Director, Ratcliffe Hicks School, University of Connecticut, Storrs, Connecticut.

Minnesota
4-H Boy and Girl Named Holstein Winners

JOAN PIERSON, 18, Lake Elmo, and MARK FLOM, 19, Kenyon, have been named state champions in the 4-H Holstein contest for 1960 in Minnesota.

They were selected outstanding 4-H Holstein club members on the basis of the herd of Holsteins they have started, the records they have kept, and their leadership. They will receive merchandise awards from the Minnesota Holstein-Friesian Association, sponsor of the annual contest, at the annual meeting of the organization.

Between them, they have won 12 championships on Holsteins, 10 reserve championships, and several dozen blue ribbons. Both have held offices of president and vice-president of their local 4-H clubs. As junior leaders they have been active in helping younger members in dairy projects.

1961 Student Honor Awards in Dairy Technology

Three seniors in Dairy Technology at the Ohio State University were honored recently during the 44th Annual Convention of the Ohio Dairy Products Association.

The title Dairy Tech Booster of 1961, and a cash award of $150, was presented R. J. KLAUSING, of Lima, Ohio. This award, sponsored by the Ohio Dairy Boosters Association Scholarship Fund, annually gives recognition to a student in Dairy Technology who has best displayed the leadership qualities expected of an ardent booster of the Dairy Industry. As president of the Student Dairy Tech Club, Mr. Klausing has led it to unusual success, and served as one of its representatives at the A.D.S.A. annual meeting in 1960.

Two Dairy Technology seniors were co-recipients of the Robert B. Stoltz Dairy Technology Award. This award also carries a cash value of $150, and is presented annually in recognition of outstanding scholastic achievements. G. S. JASCHECK, Columbus, and L. D. KNIERIEM, Elmore, Ohio, were honored by this year's award. They were recognized for their performances in maintaining superior academic records while entirely financing their own education. Both are married, and each has held one or more of the many scholarships available to students in the Department of Dairy Technology. They are student affiliates of the A.D.S.A., and were members of the 1960 Dairy Products Judging Team.

The March meetings of the Central Ohio Dairy Technology Society and the Cincinnati Dairy Technology Society were held on March 8 and 9, respectively. The featured speaker at each of these meetings was Mr. M. W. Hales, President, Cultured Products Division,

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Klenzade Products Inc. Mr. Hales's topic was How Should Cultures and Cultured Products Be Handled in the Dairy Plant? He also presided over a cultured products clinic for which products were provided by those in attendance.

The Dairy Technology Societies of Northeast Ohio, and Maumee Valley met on March 8 and 9, respectively, to hear Mr. Wayne Churchill, of the American Dairy Association discuss the topic Sales and Sales Promotion. Mr. Churchill presented the 1961 merchandising and advertising promotion of the Association, and discussed other aspects of its function as well.

The Dairy Tech Club of the Ohio State University held its annual dinner-dance at Lincoln Lodge in Columbus on February 4, 1961. Forty-two couples, including nearly all faculty members and wives, attended the affair which was partially subsidized by the Club treasury through its many enterprises. These include a Christmas Cheese sale and the preparation and serving of lunches at such Department activities as the Annual Homecoming Brunch and the Dairy Technology Conference. Special recognition was given to the Club officers, scholarship holders, the Dairy Products Judging Team, and the winners of the 1961 Student Honor Awards in Dairy Technology.

The 28th Annual Dairy Technology Conference of The Ohio State University, held February 7-9, was attended by approximately 450 dairymen from Ohio and neighboring states. Fifty-four speakers and guest speaker chairmen were included on the program, which was divided into five sections, and had as its theme Problem Solving in Science and Management.

The Fieldmen's Section occupied two full days of presentation and discussions on timely areas. Three general sessions were held, with a fourth session being devoted to separate sections for fluid milk and manufacturing milk fieldmen. The high light of the general session was a symposium on pesticide and antibiotic residue and testing problems in milk. There was a lively exchange of ideas in the area of testing programs being used and ways of assisting the milk producer. Other areas covered included discussions on silage and pasture crops and the demonstration of a new method for making automatic sediment tests on bulk tank milk.

In the section for manufacturing milk fieldmen, the general theme was public relations. A panel discused techniques available to the manufacturing milk industry for improving relations with their public. The fluid milk fieldmen's section was devoted to problems associated with bulk tanks.

The Engineering Section of the Conference emphasized the rapid advance of the Dairy Industry with respect to equipment development for automation. In general discussions on automation, it was indicated that during the next decade the industry would progress long the lines of consolidating and modifying advances already in existence. The viewpoint of industry on advantages and pitfalls of automation were presented also. Recent advances in other specific engineering areas relating to increased efficiency were discussed. Topics included developments in dairy floor and wall materials, plastic containers for milk, and welded glass lines. The latter was accompanied by several demonstrations of glass line welding. Another important phase of the Engineering Section was presentations on recent improvements in weighing and metering milk, using load cells and milk meters. The necessity of keeping track of the receipt and flow of milk within the plant was emphasized.

Mr. R. J. Sharkey, Detroit, Michigan, is shown responding to a question during the Engineering Section of the Ohio State University Dairy Technology Conference. Other speakers on the platform are (left to right), Dr. P. H. Tracy, DeLand, Florida; E. D. Weber, Cincinnati, Ohio; D. A. Seiberling, Beloit, Wisconsin; G. Andrews, Flushing, New York; R. Balvin, Cedar Rapids, Iowa; E. A. Quist, Corning, New York; and Dr. L. R. Dowd, University of Connecticut.

High lights of the Laboratory Control and Cultured Products Section included discussions of bacteriological techniques and keeping quality of Cottage Cheese, and a presentation on fluid milk defects and corrective measures. Other topics in this section dealt with cultures and problems in milk fermentations, the manufacture of cultured cream and dips, and an outline of research activities in the Department of Dairy Technology concerning antibiotics, spore-forming organisms, bacteriophage, buttermilk flavor, and lactic acid in Cottage Cheese. Automation was emphasized

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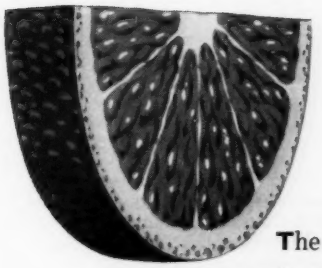

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in this section as well, by a discussion of steps for automating Cottage Cheese operations in large and small dairy plants.

A special feature of the Management Section was a clinic on complete diet products and modified milks. The production and economic factors associated with such products were discussed, during which the importance of accuracy in nutritional claims and care in cost analysis was stressed. During a panel discussion on labor relations, panelists representing labor, management and education agreed that an important problem in the years ahead is that of unemployment. The conclusion also was reached that many critical labor-management conflicts would be avoided if there were to be freer discussions by union and management representatives at the time the given problem arises, rather than accumulating all of the individual issues and delaying discussion until contract bargaining times.

Dr. I. A. Gould, Chairman of the Department of Dairy Technology, is shown reading the citation announcing the 1961 Student Honors Awards in Dairy Technology. Recipients of the awards are (left to right) George S. Jascheck and Lowell D. Knieriem, co-recipients of the Robert B. Stoltz Scholarship Award, Dr. Gould, and Ronald J. Klausing, recipient of the Ohio Dairy Boosters Association Scholarship.

In the Ice Cream Operation Section, there was considerable interest in a discussion of recent developments with regard to the Federal Ice Cream Standards. Those aspects concerned with labelling requirements and reduction of composition levels by bulky flavoring materials were stressed. A display of ice cream variegators and ice cream made from them illustrated a talk on desirable production practices for this product. A discussion of the latest developments in ice cream packages and packaging, particularly the new convenience concept in half-gallon packages, was augmented by films showing such packages in production. The place of low-conversion corn sweeteners in the ice cream industry was a topic which, together with that of obtaining good flavor quality, generated considerable discussion. The formal program was completed by discussion on the Ice Cream Audit Program and the research on the Chemical Nature of Ice Cream Flavors now being conducted by the Department of Dairy Technology. In addition, clinics on special ice cream flavors, and on chocolate ice cream samples used in the Department's ice cream flavor research were held.

Among the visiting speakers at the Conference, the following representatives of the Land Grant Colleges were present: Dr. L. R. DOWD, University of Connecticut; Dr. L. G. HARMON and PROFESSOR D. L. MURRAY, Michigan State University; and Dr. F. V. KOSIKOWSKI, Cornell University; Dr. P. H. TRACY, Professor, Emeritus, University of Illinois, and now a consultant to the Dairy Industry, also made several appearances on the program.

Ohio

Paul Luyster Awarded Prize at State Fair

PAUL LUYSTER received first prize at the Ohio State Fair for his outstanding proficiency in making Cottage Cheese. He is a member of the Barnesville Dairymen's Cooperative Association. This is the second award to this organization. Mr. Luyster's success is due to accurate control of time, temperature, and acidity during manufacturing operations. He believes that excellence in Cottage Cheese will contribute greatly to increased sales.

Earl Poling of the American Dairy Association is shown at the left presenting the certificate to Paul Luyster, as Professor Kristofferson of The Ohio State University looks on.

Completed Thesis

M.S. Degree:

ROY DALE CASSELL—Comparison of age conversion factors for Ayrshire butterfat records. West Virginia University.

USDA Announcement

Dairy Scientists in the U. S. to Participate in International Dairy Congress

Plans for U. S. participation in the Sixteenth International Dairy Congress have been announced by R. H. HODGSON, United States liaison officer for the Congress and President of the American Dairy Science Association.

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It will be held in Copenhagen, Denmark, Sept. 3-7, 1962.

In preparation for the Congress, Dr. Hodgson is inviting scientific and technical workers in the field of dairy science to submit papers to be included in the conference program and published in the proceedings. Dr. Hodgson is director of the Animal Husbandry Division, Agricultural Research Service, U. S. Department of Agriculture. Aiding him is a committee of USDA dairy specialists.

Early notification of proposed papers is essential for use of the Organizing Secretary of the Congress, Dr. Hodgson said. Scientists who wish to propose papers for the Congress are requested to notify the committee no later than May 1, 1961. Correspondence concerning proposals may be addressed to him at the Agricultural Research Center, Beltsville, Md. Information supplied should include title of the proposed paper, authors and their positions and addresses, and the section of the Congress in which the paper should be included.

Length of papers is limited to 2,000 words, according to the official Congress prospectus. Five copies of each manuscript in final form must be submitted by Sept. 1, 1961. In addition, authors must supply on separate sheets five copies of an abstract not exceeding 200 words in each of the official languages of the Congress—English, French, and German.

The Congress program lists sections on milk production, fluid milk, butter, cheese, condensed milk and milk powder, dairy machinery, ice cream, control and analysis, economy and organization, and dairying in warm countries.

The Danish National Committee of the International Dairy Federation, with the approval of the Danish government, is organizing the 1962 Congress. Organizing Secretary is P. KOCK HENRIKSEN, XVI International Dairy Congress, Raadhuspladren 3, Aarkus, Denmark. King Frederick IX is patron of the 1962 Congress.

News from Industry

Borden Foods Company Promotes Three

A. B. EREKSON, vice-president in charge of research and development for the Borden Foods Company for the past three years, has been named vice-president in charge of production. J. O. GROM, who for the past three years has assisted Erekson, will assume administrative responsibility for the division's research and development department. He will continue as an assistant vice-president of Borden Foods.

J. F. HALE, formerly assistant director of the Research Centre of the Borden Foods Company at Syracuse, N. Y., has been named technical director of research and development at the Borden Foods headquarters offices in New York City.

Borden's Acquires Caribbean Dairy

Puerto Rico Dairy, Inc., one of the Borden Company's international operations, has acquired Caribbean Dairy, Santurce, P. R. The acquisition was announced by R. L. MCCONNIE, president of Puerto Rico Dairy. It involves the purchase of Caribbean's business, machinery, and equipment, but no land. Caribbean Dairy, however, will continue to operate temporarily from its present plant.

Cherry-Burrell Installs in Belgium

The Cherry-Burrell Corporation of Cedar Rapids, Iowa, has shipped to Belgium the first continuous buttermaking system ever installed in that country.

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H. G. Hodges Joins De Laval Separator Company

H. G. HODGES has been retained by The De Laval Separator Company as a consultant to its Dairy Engineering Division.

For the past fifteen years, Dr. Hodges has been Supervising Veterinarian for the New York State Mastitis Control Program, which has national recognition for its effective work. As consultant to De Laval, he will be responsible for evaluating various milking equipment and milking methods.

H. G. Hodges

Dr. Hodges has had a lifetime of experience in dairying. He was born and grew up on a dairy farm in eastern New York State and was graduated from the New York State Veterinary College, at Cornell. He served overseas in World War I as a first lieutenant in the Veterinary Corps. Dr. Hodges was associated with the Borden Company as Field Veterinarian in charge of dairy cattle diseases and their relation to milk quality. He also served as an assistant chief veterinarian. He is active in many professional organizations. He is a member and former president of the New York State Veterinary Medical Association, and is currently New York delegate to the American Veterinary Medical Association. He is a member of the New York Association of Milk Sanitarians and chairman of its Mas-

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>
> —Russell Roberts
> Trainee Sealtest Foods
> Southern Division
> Atlanta, Georgia

CONTENTS

SECTION I states the objectives and requirements of the program.

SECTION II contains training schedules for all phases of plant operation for all types of plants. Many questions are asked for the trainee to answer before passing from one phase of training to the next, and progress reports and rating forms are provided.

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titis Sub-Committee. Dr. Hodges also belongs to the International Association of Food and Milk Sanitarians and is a member of its Mastitis Action Committee. Dr. Hodges will be located at De Laval headquarters in Poughkeepsie, N. Y.

Mrs. Dorothy McCann Returns to Thailand for Further Market Development Work Under DSI-FAS Program

MRS. DOROTHY L. MCCANN, who has been in the United States and Central America for several months, is returning to Thailand early in February to resume direction of the joint market development program of Dairy Society International and the Foreign Agricultural Service. Participating actively as third-party cooperator is the U. S.–Thai owned Foremost Dairies (Bangkok) Ltd.

Marketing Possibilities in Iran and Greece to Be Studied by DSI-FAS Dairy Products Team

Current possibilities for widening the market for dairy products in Iran and Greece will be studied by an industry-government team under the cooperative market development agreement between Dairy Society International (D.I.S.A.) and the Foreign Agricultural Service (F.A.S.) of the U. S. Department of Agriculture. Representing DSI in the survey will be C. E. STONE, former secretary-manager of the American Dairy Association of Michigan. The FAS member of the team is W. E. SCHOLZ, a marketing specialist of the Dairy and Poultry Division of the USDA.

The marketing survey is the first major activity under a new widely based Supervisory Agreement which enables the Society to establish regional headquarters in three areas from which market development activities can be administered for four continents—Europa, Asia, Africa, and South America. Greece has been named a potential Mid-East headquarters country, with Iran falling within its administrative orbit.

Dairy Technology Societies

Central Illinois—Speaker for the February 8 meeting was Henry Breejen, Sales Engineering Department, Packaging Equipment Division, Ex-Cell-O Corporation, Detroit. His topic: New Developments in Packaging Equipment Materials for Milk.

Kansas City—Subject of the February program was Sanitary Standards and Public Health. Guest speaker: Harold Thompson, Regional Milk and Food Consultant, Region 6, U.S.P.H.S., Kansas City.

Metropolitan—Program speaker at the February meeting was Francis X. Kobe, plant manager, Rockwood Chocolate Company, Brooklyn, New York, whose topic was The

Story of Cocoa and Its Applications in the Dairy Industry.

North Carolina—Case Handling Costs in Fluid Milk Plants was the program topic for the month of February, with Dr. R. L. Simmons, Assistant Professor of Dairy Marketing, Department of Agricultural Economics, North Carolina State College, Raleigh, as speaker. March meeting will feature Al Schock of Nordics Foods Company, Sioux Falls South Dakota. His topic: Cottage Cheese—The Road to Dairy Plant Profits.

New Appointments at Paul-Lewis Laboratories

E. J. BEERY has been appointed sales representative for Paul-Lewis Laboratories. According to C. B. DEDERICH, Sales Manager of the Dairy Division, he will succeed OSCAR KRAUSE, who plans to enter semi-retirement this March.

E. J. Beery

Prior to joining Paul-Lewis, Beery owned and operated the Grass Lake Cheese Factory in Shawano County, Wisconsin. He is a past president of the Wisconsin Cheesemakers Association and the Northeastern Cheese and Buttermakers Association, and served as a director of the American Dairy Association of Wisconsin. He was one of the founders of the Wisconsin Cheese Foundation and is a director of this organization. In addition, he has been active as a leader in 4-H Club work and in various farm and agricultural groups.

In his new position, Beery will represent Paul-Lewis in Wisconsin and Michigan, headquartering out of his home in Clintonville, Wisconsin.

M. L. WILLEMS has been appointed to the sales staff of the Dairy Division. According to CARL DEDERICH, Sales Manager of the Dairy Division, Willems will represent Paul-Lewis in the Midwest and South Central areas of the country.

M. L. Willems

Mr. Willems attended State Teachers College in Racine, Wisconsin, and Iowa State College in Ames, where he majored in Dairy and Food Industry. After leaving school he worked as a quality control field man for Kraft Foods, a position he held until his recent change.

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RESEARCH PAPERS

AMINO ACID AND VITAMIN COMPOSITION OF *SACCHAROMYCES FRAGILIS* GROWN IN WHEY

AARON E. WASSERMAN
Eastern Regional Research Laboratory,[1] Philadelphia 18, Pennsylvania

SUMMARY

Saccharomyces fragilis grown in a whey medium was analyzed for amino acid and vitamin contents. Qualitatively, the amino acid composition of *S. fragilis* was similar to that reported for other yeasts. The quantity of the individual amino acids, however, was greater in the whey-grown *S. fragilis*. Lysine, in particular, was present at the level of 11.14 g/16 g. N, compared to the range of 3.8 to 6.9 g/16 g. N found in other microorganisms.

An extraction procedure applied to whole, dried *S. fragilis* cells removed 40% of the total weight and 28% of the N of the cell preparation. The amino acid composition of the residual protein fraction was the same as that of the whole cells, but the amounts of the component amino acids varied from 35% of the histidine to 100% of the serine and valine in the whole cell preparation.

Thiamine, pyridoxine, riboflavin, niacin, folic acid, pantothenic acid, p-aminobenzoic acid, choline, inositol, and biotin were present in *S. fragilis* in concentrations within the ranges reported for other microorganisms.

The role of dried yeast as a source of proteins, vitamins, and other growth factors in human and animal nutrition is well established. Generally, strains of *Saccharomyces* and *Torulopsis* yeasts have been used for food purposes. In addition to baker's yeast and debittered brewers' yeast, *Saccharomyces* yeasts have been grown in molasses and in unhopped grain media as primary yeast for food and animal feed. *Torula* yeast, although capable of good growth on molasses medium, has been used extensively in the conversion of carbohydrate-containing industrial wastes into protein- and vitamin-rich products (18, 19, 23, 27).

Whey produced in the manufacture of cheese has often been treated as a waste. However, in Germany during the protein shortage of World War II, attempts were made to grow *Torula utilis* (8) and *Saccharomyces lactis* (22) on whey. The propagation procedures involving these strains of yeast were not economically practical, and the use of whey as a medium for yeast growth has not been exploited commercially. Recently, a modified procedure for the growth of *Saccharomyces fragilis* on whey has been reported (29). In addition to utilizing the waste whey, thus reducing to some extent the increasingly important problem of water pollution, the growth of yeast gives a product that might be used for animal feed and human diet supplementation.

Analyses of the various strains of yeasts grown on different media indicate that, qualitatively, the amino acid composition of the proteins and the vitamin contents are the same. Quantitatively, the individual amino acid concentrations

Received for publication August 8, 1960.

[1] Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

appear to vary only slightly within a narrow range, regardless of the strain of yeast or the medium in which the yeasts are grown (5, 10). The vitamins, on the other hand, do show a wide range of variability depending on the concentration of the particular vitamin, or precursor, in the medium, the synthetic ability of the yeast, and the growth conditions (21).

The amino acid and vitamin composition of *S. fragilis* have not been reported previously. The concentration of the individual amino acids and vitamins, however, should not vary considerably from the range of values found in the other yeasts. The data reported in this paper confirm the fact that the composition of *S. fragilis* grown in whey is similar to that of other yeast species used as a supplement in human and animal nutrition.

MATERIALS AND METHODS

Organism. The conditions for the growth of *S. fragilis* NRRL Y1109 have been described previously (29). The yeast, grown in Italian cheese whey in the pilot plant apparatus (28), was concentrated to a cream containing 15-18% yeast solids in a DeLaval separator. The yeast was washed twice with equal volumes of cold tap water and reconcentrated with the separator. The washed yeast was dried on a double-drum drier.

Nitrogen. Total nitrogen was determined by hydrolysis of the sample according to a modification of the Koch-McMeekin method (14), followed by micro-Kjeldahl distillation into boric acid.

Protein. The protein contents of the samples were calculated on the basis of N × 6.25.

Fractionation. A yeast protein fraction was prepared by treating the dried yeast according to the procedure of Roberts *et al.* (20). Five-gram quantities of yeast were suspended successively, with continuous stirring, in 150 ml. of the following solutions: 5% trichloroacetic acid for 30 min. at 5° C.; 75% ethyl alcohol for 30 min. at 40-50°; ether–70% alcohol (1:1) for 15 min. at 40-50°; 5% trichloroacetic acid for 30 min. in a boiling water bath. The suspensions were centrifuged between each solution, and the supernatant liquids discarded. The precipitates obtained after the hot 5% trichloroacetic acid treatment were resuspended in acid-alcohol, filtered, and washed on the filter with 300 ml. acid-alcohol, followed by an ether wash. The precipitates were air-dried. Three grams of a fluffy white powder were obtained from 5 g. of yeast.

Hydrolysis. Amino acid analyses were carried out on dried yeast and yeast protein preparations hydrolyzed with 6 *N* HCl (0.25 ml. acid/mg dry weight of sample). The evacuated, sealed tubes containing the acid and sample were heated in an oil bath at 110° for 24 hr. The acid was removed, and the hydrolysate dried and stored, in vacuo over P_2O_5 and soda lime.

Amino acids. The amino acid analysis was done by resin column chromatography according to Moore *et al.* (15). The dried protein hydrolysates were dissolved in citric acid buffer, pH 2.3, and aliquots placed on the short column for the determination of basic amino acids. The remaining amino acids were determined on the long column.

Vitamins. The following vitamin analyses of the dried yeast powder were carried out by the Wisconsin Alumni Research Foundation: choline (12), inositol (4), thiamine (3), vitamin B_6 (4), riboflavin (3), niacin (3), folic acid (3), pantothenic acid (16), p-amino benzoic acid (PABA) (1), and biotin (31).

RESULTS

Amino acid composition. The amino acid composition of the whole cells of *S. fragilis* is shown in Column 1 of Table 1. Quantitatively, the amino acids of

TABLE 1

The amino acid composition of *Saccharomyces fragilis*, other microorganisms, and yeast protein fractions

(The data are reported as grams/16 grams N)

	1	2	3	4	5	6	7	8
Amino Acid	*S. fragilis* whole cells	*S. fragilis* protein	12 Brewers' yeast[a] *(av.)*	4 Primary yeast[a] *(av.)*	Composite protein *(6-yeast)*	*Torula utilis*[a]	*Streptococcus fecalis*[a]	*Escherichia coli*[a]
Lysine	11.14	10.15	6.9	6.3	10.4	8.7	7.2	3.8
Arginine	7.37	7.08	5.4	4.5	5.7	7.6	3.4	4.2
Histidine	3.98	1.97	2.5	2.7	3.0	2.8	1.3	1.2
Aspartic Acid	10.40	11.16	7.2	7.2	10.2	....	7.6	....
Threonine	5.57	6.46	4.6	4.9	7.5	5.4	3.3	3.1
Serine	5.21	6.96	4.2	4.2	7.3	....	1.9	....
Glutamic Acid	15.24	13.26	10.9	10.9	7.9	14.5	10.1	....
Proline	.....	4.31	4.0	4.0	4.9	....	2.6	....
Glycine	4.24	4.63	4.0	3.9	4.5	0.4	4.6	....
Alanine	7.21	8.17	5.5	5.5	7.8	....	5.7	....
Valine	5.72	7.78	4.9	4.7	8.0	6.3	4.5	6.1
Methionine	1.57	1.24	1.3	1.4	2.0	1.8	1.9	2.0
Isoleucine	5.05	6.00	4.3	3.9	6.1	7.9	4.1	4.3
Leucine	.....	9.60	6.0	5.3	8.0	7.5	4.7	8.3
Tyrosine	4.57	3.42	3.3	3.4	4.1	....	2.2	....
Phenylalanine	5.05	5.39	3.0	3.2	4.4	5.1	2.8	2.8
Nitrogen	7.97	9.59	7.8	8.3	8.9	7.6	13.2	12.0
Reference			(6)	(6)	(6)	(13)	(25)	(2)

[a] These analyses are of whole cell preparations.

S. fragilis compare well with the average concentration of amino acids in 12 strains of brewers' yeast (Column 3), four strains of yeast grown expressly for feed or food purposes (Column 4), and *T. utilis* grown on wood sulfite waste liquor as a feed yeast (Column 6). For the most part, the concentration of the amino acids is somewhat higher in *S. fragilis*. It is interesting to note that the concentration of lysine is considerably greater in *S. fragilis* than in the other yeasts. This factor may be of importance in considering *S. fragilis* as a dietary supplement, particularly in view of the importance of lysine in nutrition.

The amino acid analysis of the whole yeast cell does not represent the composition of the true yeast protein. The protein contents of the whole cell is generally computed from the total nitrogen determination (N × 6.25). The total nitrogen, however, includes nucleic acids, free amino acids, and nonprotein nitrogen compounds such as choline and glutathione. The pure protein may

actually account for only 64-67% of the total nitrogen (9), and can be determined only after the removal of the extraneous nitrogenous matter. There is, however, no standard procedure for preparing purified yeast protein. Whole yeast cells have been treated in various ways with a number of solvents. The results of these treatments may be questionable with respect to the quantity of nonprotein nitrogen removed.

A study was made of the protein fraction of *S. fragilis*. Five-gram aliquots of the whole yeast were extracted as described in Methods. A 40% loss in solids resulted from this treatment. This protein fraction contained 9.59% of N, or 60% protein. The fraction, therefore, was not pure protein. If the nonprotein nitrogen components had been effectively removed, the remaining protein may be combined with carbohydrate or lipids, both of which are present in the yeast cell. Analysis of the yeast protein fraction for amino acid concentrations yielded the results shown in Column 2, Table 1. The differences in the amino acid concentration (based on 16 g. N) between the whole yeast cell (Column 1) and the yeast protein do not appear to be very great.

The quantity of the amino acids extracted by the treatment was calculated as shown in Table 2. Total amino acid concentrations were determined for the

TABLE 2

Total, combined, and extractable amino acid content of *Saccharomyces fragilis*[a]

Amino Acid	Total (μg.)	Combined (μg.)	Extractable % total
Lysine	266	183	31
Arginine	175	127	27
Histidine	96	34	65
Aspartic Acid	260	200	23
Threonine	138	116	16
Serine	130	126	3
Glutamic Acid	377	239	37
Glycine	105	83	21
Alanine	179	147	18
Valine	142	140	1
Methionine	39	22	43
Isoleucine	125	108	13
Tyrosine	114	62	46
Phenylalanine	125	97	22

[a] Total amino acids in 5 g. dried *S. fragilis* prior to extraction described in Methods. Combined amino acids are the residual amino acids in the 3 g. material remaining after treatment. Extractable amino acids include the free amino acid pool, polypeptides, and soluble proteins. They are determined as the difference between the total and combined amino acids.

5 g. of yeast prior to extraction. The yeast protein amino acids, or combined amino acids, are the residual amino acids in the 3 g. of material remaining after extraction. The term combined amino acids is used to include any amino acids that may be complexed with carbohydrates or lipids, as well as the pure protein material. The differences between the total and combined amino acid values are the extractable amino acids. This fraction should include the components of the free amino acid pool, polypeptides, and soluble proteins.

The range of concentration of the extractable amino acids of *S. fragilis*, grown in whey medium under the given conditions and extracted as described,

varies considerably, from essentially no serine or valine to 65% of the total histidine present in the yeast. Approximately 45% of the methionine and tyrosine, and about one-third of the lysine and glutamic acid were extracted. The other amino acids were soluble in the treatment solutions to the extent of 13 to 27% of their total concentrations.

Vitamin composition. The vitamin composition of *S. fragilis* is shown in Column 1, Table 3. The concentration of the vitamins in *S. fragilis* compares well with that reported for other yeasts (Table 3).

DISCUSSION

S. fragilis can be grown in a whey medium to contain 50% protein. The strain of yeast and the medium on which it was grown have no influence on the amino acid composition of the protein and only some slight effect on the concentration of the various amino acids. The amino acids in *S. fragilis* do not differ from those found in other yeasts grown in various media, and the amounts of the amino acids fall, for the most part, within the range observed for other yeasts. *S. fragilis*, in common with most of the yeasts used for food purposes, is low in S-amino acids. *T. utilis* grown in sulfite liquor also was low in S-amino acids and had to be fortified with methionine to give values comparable to casein (11) in feeding tests.

Analysis of the whole yeast cells for amino acid composition can yield misleading information about the protein structure. The quantity of some amino acids may be reduced through reaction with carbohydrates during acid analysis. On the other hand, the apparent concentration of the amino acids in the yeast protein may be greater because the free amino acids, and those liberated from polypeptides by acid hydrolysis, are also included in the determination of amino acids. The extraneous nonprotein nitrogen and carbohydrate compounds should be removed from the yeast to avoid these errors. There is no standardized method for accomplishing this, and many procedures have been used (5). The method of Roberts *et al.* (20) has been used for the extraction of *Escherichia coli* and the materials removed by the various solvents were described in detail. Applying this procedure to *S. fragilis* removed 40% of the weight of the cell preparation and 28% of the nitrogen. The remaining material was not all protein; it may be protein combined with carbohydrate or lipids. The extractable amino acids are of interest in view of their possible importance in protein synthesis. For instance, serine and valine are present entirely in the protein, or combined fraction, whereas 65% of the histidine in the cells is present as extractable amino acid. The other amino acids are present in the extractable fraction in varying concentrations. The total nitrogen contents of the yeast will vary. *S. fragilis* growing in whey alone may have a nitrogen content of 4.9%, whereas in the media containing 1% $(NH_4)_2SO_4$, the growing cells may contain 8.2% N (30). Since the true protein fraction of the yeast will have the same amino acid composition and concentration under all conditions in which the yeast can remain healthy, only the amount of the amino acids in the extractable fraction can vary when the external N source is changed. The quantities of the

TABLE 3

Vitamin composition of *Saccharomyces fragilis* and other yeasts

Vitamin	*S. fragilis*	*Torula utilis*	*Torula utilis*	*Torula utilis*	Brewers' yeast	Bakers' yeast	Whey
Thiamine *(μg/g)*	24.1	6–53	5.3	7–42	104–250	11–60	4–15
Pyridoxine *(μg/g)*	13.6	35	33.0	28–47	23–40	25–39	.5–15
Riboflavin *(μg/g)*	36.0	26–62	45.0	24–61	25–80	25–80	5–68
Niacin *(μg/g)*	280.0	210–535	417.3	375–690	300–627	293–482	3–22
Folic Acid *(μg/g)*	5.8	4–31	21.0	.....	19–30	25–34	0–1
Pantothenic Acid *(μg/g)*	67.2	86–180	37.0	.....	72–86	150–280	22–90
p-Aminobenzoic Acid *(μg/g)*	24.2	17–21	.....	11–40	15–40	24–167	.....
Biotin *(μg/g)*	2.0	1.1–1.9	2.3	1.1–1.7	1.1	0.8–2.4	.03–1.5
Choline *(mg/g)*	6.7	.....	.....	.....	.....	.....	0.5–4
Inositol *(mg/g)*	3.0	3.5	.....	2.8	.....	.....	0.5–6
References		(10)	(13)	(23)	(17)	(17)	(7)

amino acids in the extractable fraction may depend on the yeast strain, the composition of the medium, or the age of the yeast (24).

It is of interest to note that in the column chromatography of the yeast protein, or combined, amino acids, a small peak appeared in the 35th 2-ml. fraction, before any of the amino acids were eluted. The material in this peak gave a red color upon addition of the ninhydrin developing reagent. A similar peak has been isolated and identified as levulinic acid in acid hydrolysates of bean and potato fractions by Zacharius and Talley (32). The identity of the yeast protein material has not been established. This peak was not observed in the acid hydrolysates of whole dried yeast. The extraction treatment given to the yeast apparently made the observation of this material possible by (1) concentrating the material so the small quantity present could be detected, or (2) forming or exposing a precursor compound that yielded the unknown on acid hydrolysis.

Although the vitamin composition of the yeasts appears to be constant, the concentration of the vitamins will vary depending on the yeast strain, composition of the medium, or cultural conditions. Brewers' yeast contains more thiamine than bakers' yeast, as normally produced, but enriched bakers' yeast with considerably more thiamine has been produced commercially (17). Since it is known that yeast absorbs thiamine from the medium, this enrichment can be accomplished by the addition of thiamine to the growing yeast, or to the spent medium of nonproliferating yeast (26). Whey contains very little thiamine (Table 3); therefore, the contents of vitamin B_1 in *S. fragilis* could be expected to be low. Although the vitamin value is comparable to other yeasts, *S. fragilis* could be enriched by the addition of thiamine to the medium.

A further cause of reduced vitamin concentration is the yeast yield. Increased yeast yields result in less vitamin per unit cell (21). It is possible, therefore, that under the conditions in which the *S. fragilis* was grown on whey for maximum yields, greater vitamin contents could not be achieved.

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WHEY UTILIZATION. V. GROWTH OF *SACCHAROMYCES FRAGILIS* IN WHEY IN A PILOT PLANT

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SUMMARY

Saccharomyces fragilis was grown in whey medium in a pilot plant study. A flow diagram of the process is given and the equipment described. The oxygen absorption rate (OAR) of the propagator was determined by the sulfite oxidation method. Eighty per cent of the OAR found necessary in the laboratory to supply the oxygen demand of yeast growing under similar conditions could be obtained by modifying the propagator. The yeast yield was 75% of the theoretical value. The dried yeast product is a bland powder with a pleasant, cheese-like flavor.

Whey, a by-product of cheese-making, can be used as a medium for the rapid production of a yeast suitable for the supplementation of human and animal diets. Wasserman, Hopkins, and Porges (6) reported maximum yeast growth in 4 hr., using raw whey and an inoculum of *Saccharomyces fragilis* equal on a dry weight basis to 25% of the weight of the whey sugar. High yields were dependent on the supply of sufficient oxygen to satisfy the demand of the yeast. Under the given growth conditions, the yeast required about 570 mg. oxygen/gallon medium/minute (4.75 m*M* O_2/L/min) at their peak demand period (4, 5). The yeast can only utilize dissolved oxygen, and the relation between yeast yield and the oxygen absorption rate of the propagation equipment has been reported (5).

The data reported in this paper show that the growth of *S. fragilis* in whey medium in a pilot plant operation of approximately 800-gal. volume could be successfully based on the results of the 500-ml. and 15-liter laboratory propagators reported by Wasserman *et al.* (4, 6, 7).

METHODS AND MATERIALS

S. fragilis, a lactose-utilizing yeast, was grown in raw Italian cheese whey obtained through the courtesy of the M. Maggio Company, Philadelphia, Pa. The whey was supplemented with 0.5% quantities of $(NH_4)_2SO_4$, K_2HPO_4, and dried Brewers yeast. The pH of the medium after the addition of the salts was approximately 5.5, which is within the optimum pH range for maximum yeast yields. The conditions for growing the yeast were the same as those previously described for *S. fragilis* growth in the laboratory propagators (4, 6, 7).

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Propagation apparatus. A steel storage tank, 5 ft. in diameter and 12 ft. 8 in. high, was converted into a yeast propagator. The agitation-aeration unit, designed and constructed by the Walker Equipment Company,[2] was a six-blade turbine suspended from the top of the tank and powered by a 7½-h.p. motor. The turbine rotated at 300 r.p.m. In operation, the power supplied by the motor was inadequate, but a more powerful motor could not be used because of difficulties in keeping the medium in the tank under the described conditions. A Spencer turbo-compressor,[2] rated at 360 c.f.m. at 5 p.s.i. gauge, supplied air through jet orifices mounted on a sparger ring beneath the agitator. Temperature control of the medium was effected by passing cold water (40° F.) through 118 ft. of copper tubing (1½ in. o.d.) arranged in a coil within the tank.

Although the tank was rated to contain 1,600 gal., only 700-800 gal. of medium were actually used in the propagation of the yeast, leaving about 50% of the tank capacity as head space to accommodate the increased volume of the emulsion created by aerating and agitating the medium.

Analytical determinations. The oxygen absorption rate (OAR) of the propagation equipment was determined by a modification (2) of the sulfite oxidation method of Cooper, Fenstrom, and Miller (1).

The course of the yeast growth was followed by periodic determinations of the dry weight of the yeast, and the disappearance of lactose. A sample of the yeast suspension was taken from the middle of the tank and the yeast removed by centrifugation. The dry weight of the yeast was obtained after washing the cells once with distilled water, and drying overnight at 105° C. Lactose was determined by the method of Stiles, Petersen, and Fred (3) on the supernatant medium. For immediate information of the progress of the yeast growth, cell counts were made with a hemocytometer. Although it was known that cell counts were of doubtful accuracy, the rapidity of the determination made it a useful qualitative test.

RESULTS

OAR determination. Before an attempt was made to grow yeast in the propagator, the OAR of the equipment was determined to assure solution of sufficient oxygen for maximum growth. Eight hundred gallons of 1 *N* sulfite solution was placed in the propagator and the agitator blades were set at 25-in. diameter. The air throughput was 360 c.f.m.; lower air rates could not be used because the resistance of the solution was sufficient to stop the motor. Under the above described conditions, the OAR was 3.4 m*M* O_2/L/min (418 mg. O_2/g.p.m.). Since the peak O_2 demand of the yeast was 570 mg. O_2/g.p.m. (4), only 72% of the required oxygen was dissolved by the equipment. To increase the OAR the apparatus was modified by reducing the diameter of the agitator blades to 23 in. and installing four steel baffles 90 in. long and 8 in. wide on the walls of the tank. The OAR rose to 4.0 m*M* O_2/L/min. Although this value was still below the O_2 requirements of the yeast, the apparatus could not be

[2] It is not implied the USDA recommends the above company or its product to the possible exclusion of others in the same business.

modified further at this time, and the yeast propagations were undertaken with the realization that maximum yields might not be achieved.

Yeast propagation. The flow diagram for a simple yeast propagation operation is shown in Figure 1. The raw, unheated whey and the solid medium ingredients were mixed in the propagation tank, and the yeast inoculum—equal in dry weight to 25-30% of the weight of the whey lactose—was added. The inoculum may be grown in increasing volumes of whey or, as in this case, it may

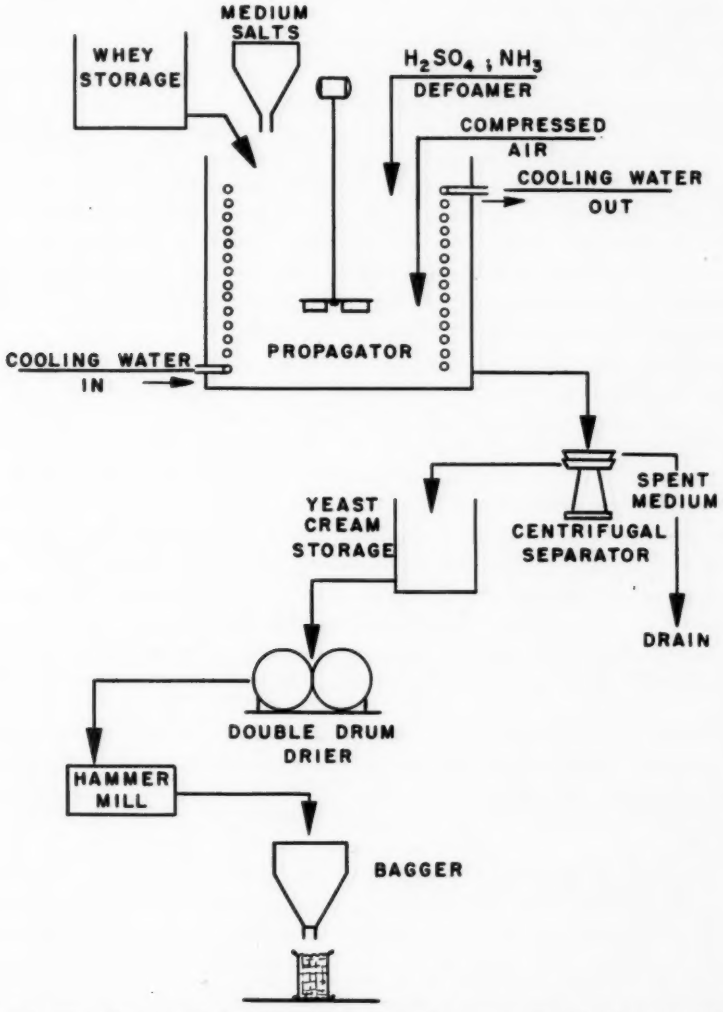

FIG. 1. Flow diagram of the pilot plant process for propagating *Saccharomyces fragilis* on whey medium.

be a concentrated yeast cream from a previous tank. The pH of the propagation was maintained at about 5.5 by the addition of concentrated H_2SO_4 or NH_4OH. At the conclusion of the experiment the yeast suspension was passed through a DeLaval separator [2] to concentrate the yeast to a 15-18% slurry. The yeast cream can be washed by suspension in water and reseparation as many times as necessary. The yeast cream was fed to a double drum dryer operated at a steam pressure of 85 p.s.i. gauge and rotating at 12 r.p.m. The dried yeast flakes were carried by a worm conveyor to a hammer mill to be pulverized, then to a bagging apparatus.

The product was a light cream-colored powder with a bland, slightly cheese-like flavor. The intensity of the flavor can be controlled by the number of washes the yeast is subjected to prior to drying.

Data for two typical batches of yeast grown as described are presented in Table 1, and the course of a representative propagation is shown in Figure 2. Although the volumes of medium used were different (530 gal. for Batch 1 and 800 gal. for Batch 2), all of the available lactose in Batch 2 had been utilized within 4 hr. and less than 0.5% still remained in Batch 1 at this time. The maximum yeast growth in both runs was achieved in 4 hr. Propagation beyond this time led to no further increase in yeast yield and could even be conducive to destruction of some of the yeast already formed.

Laboratory data indicated that 0.55 lb. of dry yeast could be obtained per pound of lactose used (6). Thus, from the 182 lb. of sugar in Batch 1, 100 lb. of dried yeast were anticipated, but 74 lb. were actually obtained. In Batch 2, the 242 lb. of lactose should yield 133 lb. of yeast, and 105 lb. were actually found. A yeast yield of approximately 75% of theory, or about 0.42 lb. per pound lactose, was obtained.

The factors involved in the decreased yeast yield were not determined. However, since laboratory-scale propagations in Italian cheese whey media resulted in yeast crops approximating the theoretical yields, the similar medium components in the 700-gal. propagator could be expected to yield an equally large crop of yeast. A direct correlation between the OAR of a propagator and the yeast yield has been demonstrated (5); therefore, if the quantity of dis-

TABLE 1

Representative balance sheet for the growth of *Saccharomyces fragilis* in whey medium in pilot plant equipment

	Batch 1	Batch 2
Volume whey *(gal.)*	450	600
Lactose (4.86% of whey) *(lb.)*	182	242
Lactose disappearing *(lb.)*	182	242
Volume seed yeast *(gal.)*	81	150
Weight seed yeast *(lb.)*	66	110
Final volume in tank *(gal.)*	530	800[a]
Gross weight of yeast yield (dry) *(lb.)*	140	215
Net weight of yeast yield (dry) *(lb.)*	74	105
Theoretical yeast yield (55% of sugar weight) *(lb.)*	100	133
% Theoretical yield	74	79

[a] Fifty gallons of water added accidentally.

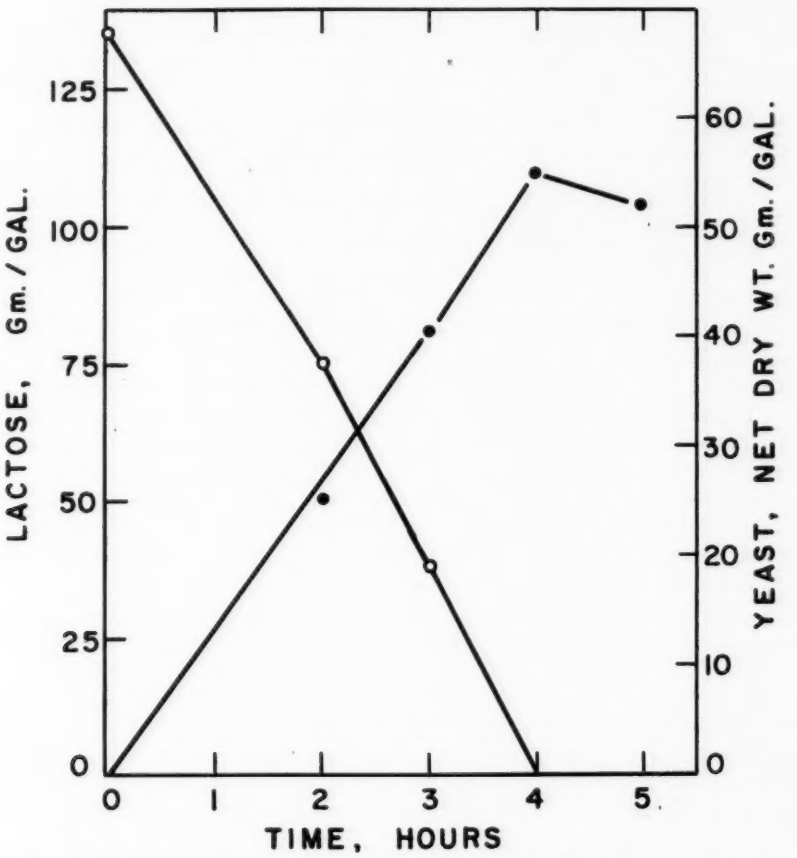

FIG. 2. Lactose utilization and *Saccharomyces fragilis* production in pilot plant Batch 2 (see text). Lactose ○———○; Yeast (net dry weight) ●———●.

solved oxygen in the 700 gal. of medium were only 80% of the yeast requirements, the observed decrease in yeast yield might be expected.

To produce 1 lb. of dried yeast, 2.45 and 2.3 lb. lactose were required in Batch 1 and Batch 2, respectively. Approximately 0.27 lb. of each of the other medium ingredients was used per pound yeast.

The data indicate that whey can be used as a medium for the batch production of yeast in good yield. Increased yields might be expected with a more efficient oxygen-dissolving system and conversion of the batch process into continuous or semicontinuous operation.

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QUANTITATIVE DETERMINATION OF LACTASE

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SUMMARY

A simple and quick assay is described for the determination of the lactase activity of crude enzyme preparations. Results are available in 2 hr. from as little as 2 mg. of material. Fifteen minutes' incubation at 37° C. is used for the enzymatic reaction on a pure lactose substrate. The lactase activity is related to the amount of glucose released by the hydrolysis, as determined by an enzymatic micro-method. The effects of substrate concentration, pH, temperature, and related factors are investigated. The assay procedure was advantageously employed in a preliminary quantitative screening of lactase-producing organisms which could utilize whey in their growth.

Lactase, the enzyme that hydrolyzes lactose to glucose and galactose, is of potentially great importance to the dairy industry. In the course of establishing a screening program to find the best microbial sources of the enzyme, it became apparent that a rapid, but sensitive, lactase assay was necessary.

The few methods cited in the literature for testing commercial lactase are quite lengthy and complicated. In each, a 4-hr. hydrolysis step is followed by chemical determination of the hydrolysis products. The lactase acts upon a substrate of 30% condensed skimmilk in two of the methods (5, 9) and upon a 10% lactose solution in the other (7). The resulting mixture of reducing sugars is extremely difficult to analyze, particularly in the presence of condensed skimmilk components. Potter and Webb (5) chose to determine the intensity of the molybdenum blue color caused by the glucose-galactose mixture and reported the degree of lactose hydrolysis.

This paper describes some preliminary work on the production of crude lactase and presents a procedure by which small amounts of the crude enzyme preparations can be accurately analyzed in less than 2 hr. The enzyme reacts upon pure lactose for 15 min., then the hydrolysis products are analyzed for glucose by the specific enzymatic technique developed by Salomon and Johnson (6).

MATERIALS AND METHODS

Propagation of organisms. Lactase is generally found in microorganisms growing in milk or other products containing lactose. Many organisms, therefore, were isolated from such sources. These were propagated on a supplemented whey medium prepared as follows:

Cheese whey was autoclaved 15 min. at 15-lb. pressure and the precipitated proteins removed by filtration through cheese cloth. The clear solution was adjusted to pH 7.0 with concentrated KOH and again autoclaved. The precipitated residue was removed by filtration through coarse paper. The following materials were added to the clear solution:

$(NH_4)_2SO_4$	—	1.0% (w/v)
K_2HPO_4	—	0.5% (w/v)
Dry yeast extract	—	0.1% (w/v)

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The medium was adjusted to the desired pH and autoclaved in Fernbach flasks in 500-ml. volumes. These were inoculated with suspensions of cells washed from nutrient agar slants containing 0.5% lactose. The cultures were agitated 18 hr. on a reciprocating shaker at 28° C. The heavy growths were centrifuged, the cellular residue washed with distilled water and recentrifuged.

TABLE 1

Optimum temperature for lactase activity

° C.	μM glucose liberated
20	13.9
25	24.4
30	33.4
37	46.7
45	41.2

Preparation of crude lactase. Lactase is an endoenzyme; therefore, whole cells exhibit very little lactase activity. It is necessary to treat the cells to release the enzyme. By destruction of the cell permeability with acetone, the total lactase activity is quickly made available for assay purposes. The centrifuged cells were reconstituted in 200 ml. distilled water and slowly added to 400 ml. cold acetone with constant vigorous mixing. After 15 min. in an ice bath, with occasional stirring, the treated cells were filtered through paper, washed first with 200 ml. cold acetone and then with 50 ml. ethyl ether. The crude material was air-dried on the filter paper and refrigerated in a desiccator until assayed.

Yields of the crude enzyme preparations varied considerably. Many of the organisms were well adapted to growth in the whey medium and yielded many cells in 18 hr. A few did not. However, since only 10-50 mg. of a preparation was needed for the lactase assay, sufficient quantities were obtained.

Enzyme assay. The procedure consists of two steps: (a) hydrolysis of pure lactose by the test preparation and (b) the micro-determination of glucose in the hydrolysis products.

1. Lactose hydrolysis. A tentative lactose hydrolysis method was followed before the standard assay conditions could be established. The enzyme reaction is performed in test tubes in a water bath at 37° C. One milliliter of a 5% (0.139 *M*) lactose solution in 0.1 *M* potassium or sodium phosphate buffer of pH 6.3 and 0.8 ml. of the buffer solution are mixed and equilibrated in the bath. Two-tenths milliliter of a distilled water suspension of the dried lactase (10 mg. per milliliter) is added, bringing the total test volume to 2.0 ml. After a 15-min. incubation period, the activity is stopped by heating the tubes in a boiling water bath for 5 min. The cellular residue is removed by centrifugation, and the supernatant is analyzed for glucose.

2. Glucose determination. The composite reagent of Salomon and Johnson (6) is used for the determination of glucose. To 700 mg. *o*-tolidine dihydrochloride in a few milliliters of water add 150 ml. 2 *M* acetate buffer of pH 4.1-4.2. Dissolve 200 mg. glucose oxidase and 15 mg. horseradish peroxidase in a few

milliliters of buffer (both obtainable from Sigma Chemical Company, St. Louis, Missouri.)[1] Add to the *o*-tolidine solution and make up to 300 ml. with the buffer. Filter to remove turbidity and store at 5° C.

In the presence of glucose, the following reactions occur:

$$\text{Glucose} \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{reduced dye} \xrightarrow{\text{peroxidase}} H_2O + \text{oxidized dye (blue color)}$$

TABLE 2

Lactase activity of various organisms

Organisms	Lactase units/mg dry weight
Bacterium A	7.56
Bacterium B	13.4
Yeast A	14.25
Yeast B	19.8
Aerobacter cloacae	2.89
Escherichia coli	5.0
Saccharomyces fragilis	2.2

To determine the concentration of glucose in the lactase-hydrolyzed lactose solutions, 2 ml. of 1:20 and 1:40 dilutions of the supernatants are placed in test tubes. At the same time, a standard curve is prepared by diluting a glucose stock solution (1 mg/ml) to cover a range of 10-100 γ glucose per 2 ml. One and a half milliliters of composite reagent is added to all the tubes, which are then incubated at room temperature (25 ± 2° C.). Color development in the samples is visually compared to the standards at intervals. If the blue color developed is considerably deeper than the most concentrated standard, higher dilutions of the hydrolyzed supernatant are necessary. After 1 hr. the solutions are transferred to cuvettes and read in a spectrophotometer at 630-635 mμ. The amount of glucose present is determined from the standard curve.

The Salomon and Johnson composite reagent gave consistent color development at 25° C., but incomplete color formation has been noticed at 30° C., especially in the absence of light. A new standard curve should be prepared for each new batch of composite reagent. Several reference standards should be included in each assay.

RESULTS AND DISCUSSION

Several factors of importance in the study of enzyme reactions were investigated in establishing conditions for the lactase assay procedure. The standard lactase preparation used was an unidentified bacterium isolated from a milk agar plate exposed to air. The organism grew well on whey medium and produced lactase of high activity.

Effect of substrate concentration. The rate of the lactase reaction was studied as a function of the lactose concentration. The reaction mixture con-

[1] It is not implied the USDA recommends the above company or its product to the possible exclusion of others in the same business.

sisted of 5 mg. crude enzyme in several concentrations of lactose in 0.1 *M* sodium phosphate buffer, pH 7.0. The results plotted in Figure 1 are for micromoles glucose liberated by 5 mg. lactase in 30 min.

Effect of enzyme concentration. In enzyme assay procedures it is desirable that the relationship between the quantity of enzyme used and the amount of product released also be linear. Figure 2 shows this. Distilled water suspensions of the acetone-dried cells were used to hydrolyze solutions of 25 mg. pure lactose per milliliter under the conditions described. Saturation of the enzyme within this range of substrate did occur. From these data the choice of 1 mg. enzyme per milliliter of assay solution was justified.

Effect of time. A straight-line relationship between the time allowed for lactase activity and the amount of lactose hydrolyzed was also desired. To ascertain whether this was achieved under standard assay conditions, a time

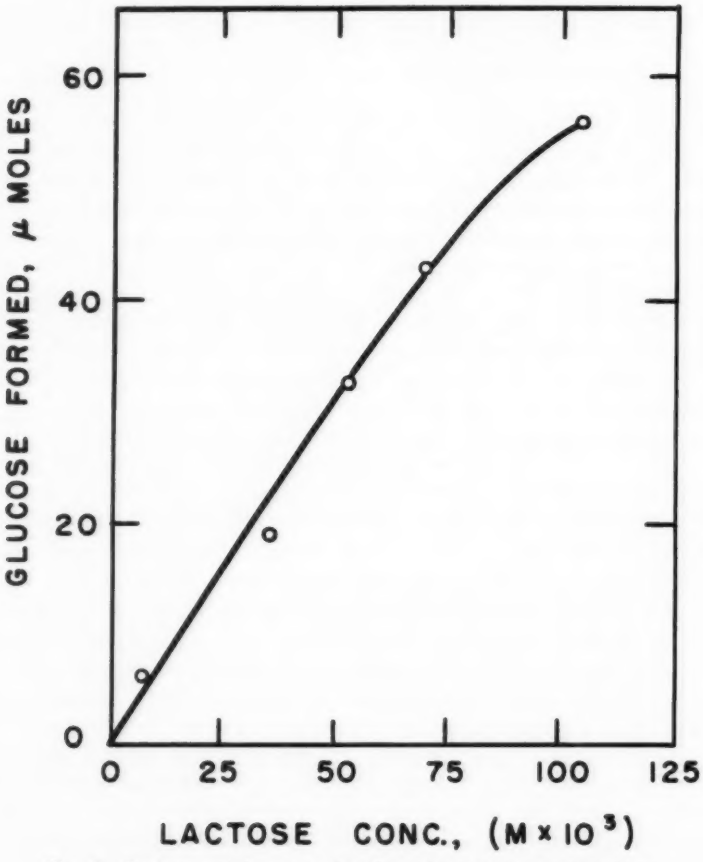

FIG. 1. Lactase activity as a function of substrate concentration.

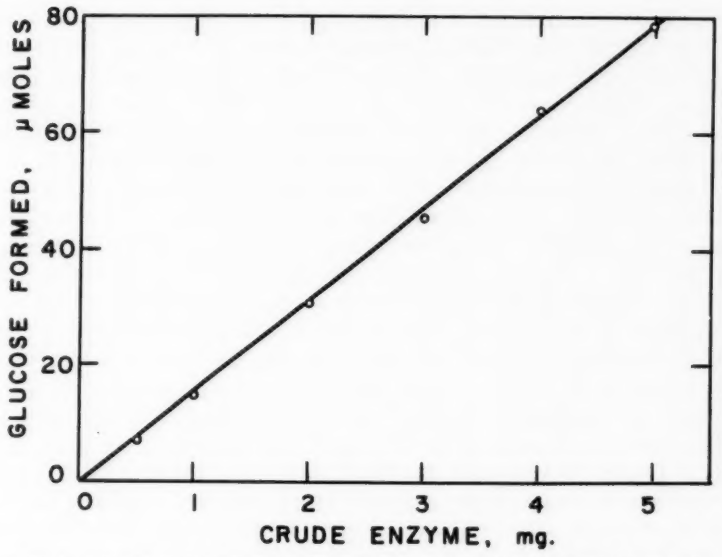

FIG. 2. The effect of enzyme concentration on the rate of lactose hydrolysis.

curve was run using 1 mg. enzyme per milliter of assay solution. Samples were incubated, quickly inactivated, and measured for glucose formation at frequent intervals during a 2-hr. period. Figure 3 is a plot of these data. Although the rate of hydrolysis was linear during a 1.5-hr. period, it was decided to determine the activity of the enzyme during the initial 15-min. period, when the rate

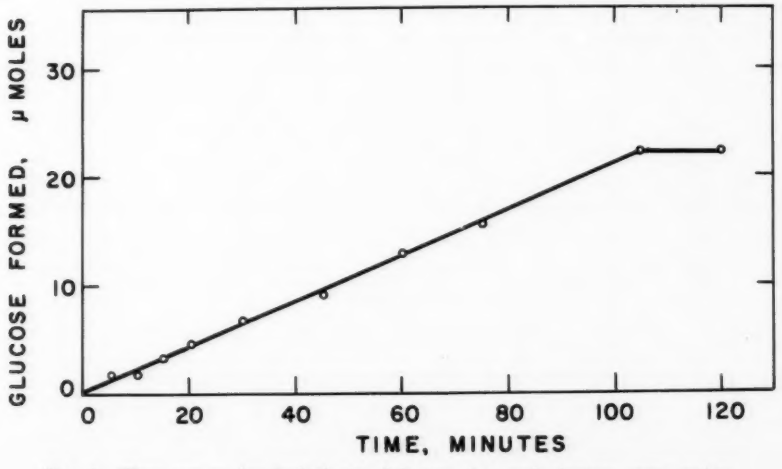

FIG. 3. Time course for hydrolysis of lactose by crude lactase preparation.

more closely represented the true reaction velocity. This would be of importance with enzyme preparations of greater activity.

Effect of temperature. The effect of temperature on the rate of lactase activity was investigated. Temperatures studied were those easily available in the laboratory. The lactose and buffer components of the reaction mixtures and the cell extracts were equilibrated separately at the selected temperatures for 30 min. before mixing and timing the assay. Results given in Table 1 point to the selection of 37° C. for the standard assay conditions. The decline in enzymatic activity at 45° C. may possibly indicate heat inactivation of the enzyme.

pH and buffer. The optimum pH for lactase varies with the enzyme source. Cajori (1) reported an optimum of 5.4 to 6.0 for the intestinal lactase of dog. Cohn and Monod (2) selected a pH of 7.0 for *E. coli* lactase (β-galactosidase) activity, while Wallenfels *et al.* (8) observed maximum at pH 7.3.

The optimum pH for the enzyme preparation used in these experiments was ascertained by using 0.1 *M* potassium and sodium phosphate buffers of various pH levels in the assay procedure. The 5% (0.139 *M*) lactose substrate and the lactase suspension were prepared in distilled water for these tests. Figure 4 shows that maximum hydrolysis occurred with a pH range of 6.3 to 6.6. Since activity was sharply inhibited on either side of pH 6-7, a buffer of pH 6.5 was selected for the standard assay.

It has been observed previously that the presence of the potassium ion strongly activated *E. coli* lactase (2, 8). This was also found to be true with

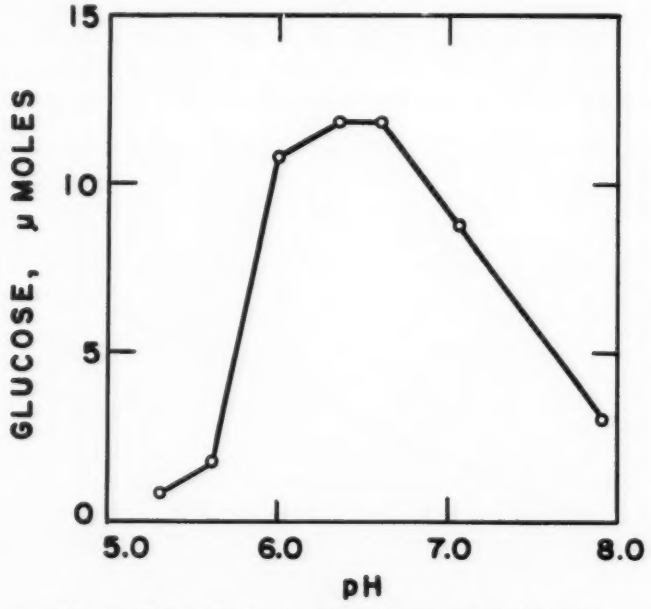

FIG. 4. Effect of pH on lactose hydrolysis by crude lactase.

the enzyme preparations tested in this study. When lactase preparations from two yeast and two bacteria were compared in 0.1 *M* sodium phosphate and 0.1 *M* potassium phosphate buffers, the activity in potassium phosphate was almost three times as great.

As a result of the above studies the hydrolysis conditions for the standard assay of lactase were selected. One milliliter of a 5% (0.139 *M*) lactose solution and 0.8 ml. 0.1 *M* potassium phosphate buffer of pH 6.5 are mixed and equilibrated in a 37° C. water bath. Two-tenths milliliter of lactase suspension is added. After 15 min. at 37° C. the enzyme action is stopped by heating for 5 min. in a boiling water bath. The centrifuged supernatant is analyzed for glucose.

A unit of lactase is defined as the amount of enzyme which will liberate 1 μM of glucose from lactose in 15 min. at 37° C.

Pazur (4) reported that lactose hydrolysis by lactase results in the formation of varying quantities of oligosaccharides in addition to glucose and galactose, depending on the conditions of the hydrolysis. The effect of oligosaccharide formation in this determination was not investigated. It was assumed for the purposes of achieving a simple and rapid assay that a measure of the amount of glucose formed was also a measure of the degree of lactose hydrolysis.

Use of standard assay in screening lactase producing organisms. A routine quantitative screening procedure utilizing the assay was initiated to detect lactase producing organisms that could grow on the modified whey substrate. Both known and unidentified lactose-utilizing bacteria were grown on the supplemented whey medium under the conditions described. These were not the best growth conditions for each strain, so yields often were quite small. However, even as little as 2 mg. of the acetone-dried lactase could be assayed. Yield of cells, therefore, was of no consideration in prejudging enzyme activity. After a potent lactase producer is revealed, its optimum growth conditions can be determined.

Approximately 60 organisms were investigated in this preliminary study. Of these, 30 were able to grow on a whey medium and produced enough cell material to be assayed at 1 mg. per milliliter concentration. During the assay test all preparations that gave only a faint blue color in the 20-fold dilution were eliminated from further consideration. The enzyme activity of these organisms was less than one unit per milligram dry weight.

Several organisms not yet completely identified give promise of being excellent producers of lactase from whey. These are compared with lactase similarly prepared from known organisms in Table 2.

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INTERFACIAL TENSIONS OF LIPOLYZED MILK FAT–WATER SYSTEMS [1]

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SUMMARY

The average interfacial tensions (in dynes per centimeter) of control and lipolyzed milk fat–water systems were, respectively: extracted fat—5.4, 4.0, after removal of free fatty acids—5.9, 2.7, after removal of acetone insoluble lipids—11.8, 5.4, and after removal of monoglycerides (as part of 80% ethanol-soluble lipids)—24.4, 22.7. It appears that monoglycerides decreased interfacial tension to some extent.

Monoglycerides have been shown to be present in lipolyzed milk fat and the presence of diglycerides has been indicated (15-17). Herrington (14) has suggested that the effects of these two classes of surface active compounds should not be overlooked as contributing, along with free fatty acids (FFA) (21), to the reduction of surface tension occurring during the lipolysis of milk fat. Duthie and Jensen (9) found that additions of pure monoglycerides to milk reduced surface tension. Mono- and diglycerides have been reported to be much more effective than either FFA or diglycerides alone in reducing the surface and interfacial tension of lipid systems other than milk fat (7, 10). It should not be overlooked that the surface activity of FFA in milk would be increased by the presence of fatty acid salts (2).

Although monoglycerides are present in lipolyzed milk fat and are effective in reducing surface tension of milk, the effect of these compounds on the interfacial tensions of milk fat systems has not been documented. While few data exist on interfacial tension of milk fat systems (3, 20), numerous investigations on interfacial tension between aqueous solutions and other fats are available (1, 4, 7, 10-12). For example, Feuge (10) noted that diglycerides in the presence of monoglycerides were, on a weight basis, less than one-hundredth as surface active as monoglycerides. A concentration of as little as 1% of monoglycerides in an oil phase lowered the interfacial tension to approximately one-half of that observed when no monoglycerides were present. A concentration of 6% monoglycerides lowered the interfacial tension approximately to zero.

In a natural emulsion such as milk, it would be extremely difficult to measure the interfacial tension of the milk fat globule–serum system. Therefore, to measure the surface activity of milk constituents, the use of a simplified system, for example, butteroil-water, is necessary. A study of lipolyzed milk fat–water

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systems, even though these are admittedly different from the fat globule–serum system in milk, should yield information on the effects of the surface active compounds released by lipolysis. Several of these substances, e.g., FFA and monoglycerides, can be removed by various methods. The purpose of this study, therefore, was to determine the effects of successive removal of several surface active substances, either present in milk fat or derived from lipolyzed milk fat, on the interfacial tension of milk fat–water systems.

EXPERIMENTAL METHODS

Preparation of samples. Homogenized, pasteurized 40% cream and raw milk were mixed in equal portions and stored at 4° C. for 48 hr. to provide a source of lipolyzed milk fat. Control samples were prepared by pasteurizing the same raw milk before mixing it with the cream; no storage was involved. The fat was recovered by silica gel extraction, using a 9:1 chloroform-ethanol mixture as the solvent (13, 16). The solvent was removed at reduced pressure with a flash evaporator, with the probable loss of some lower FFA. FFA were removed from an ether solution of the fat with the ion exchange resin IRA 400 (5). Monoglyceride extracts were isolated by dissolving the fat in heptane (20% by weight) and partitioning it with 80% ethanol (5). Since phospholipids exert an influence on interfacial tension, these compounds, together with other lipids insoluble in acetone, were removed from the extracted fat by acetone precipitation, although it was found that the 9:1 chloroform-ethanol extraction mixture only partially eluted the phospholipids from milk.

Analytical methods. Fatty acids were determined by titration of an aliquot of the fat, and monoglycerides by the method of Jensen and Morgan (16). Lipid phosphorus determinations were made as described by Smith *et al.* (19). Interfacial tension was measured in 600-ml. beakers, with the samples maintained at 56.5° in a constant temperature water bath, using a Cenco duNouy tensiometer, which had been previously calibrated to read directly in dynes per centimeter (2). For each determination, the beaker was filled to a height of several centimeters with tempered distilled water, the ring immersed in the water, the tempered fat sample carefully poured upon the water surface, and the interfacial tension measurement taken within 2 min.

Sample treatment. Control and lipolyzed fat samples were subjected to two sequences. Sequence I, in which five separate control and lipolyzed samples were employed, consisted of (a) analysis of the extracted fat for FFA, monoglycerides, and interfacial tension, (b) removal of FFA followed by determination of fatty acids, (c) removal of acetone insoluble lipids, and (d) removal and analysis of monoglycerides. Each step was followed by an interfacial tension measurement on the fat sample. The fat was separated from the water with a separatory funnel, after making interfacial tension measurements. Sequence II, in which three samples were employed, consisted of the same procedure as I, except that the acetone insoluble lipids were removed prior to the FFA. Sequence II was carried out to determine if the presence of acetone insoluble lipids exerted an effect on the surface activity of the FFA. The removal of

FFA after the removal of monoglycerides, while obviously desirable, was not undertaken, because Borgstrom (5) has observed that FFA interfered with the partition of monoglycerides between heptane and 80% ethanol.

Two additional studies were undertaken to explore the possible influence of 80% ethanol soluble lipids and of purified milk monoglycerides on the oil-water interface. Ethanol soluble lipids were added to the heptane fraction at the level of 0.35%. Milk monoglycerides were concentrated by silica-gel column chromatography (6, 18) to a purity of about 80% and added to the heptane fraction at a level of 0.69 m*M* per 100 g. fat. Interfacial tension measurements were made before and after the additions, as described above. Diglyceride fractions were not studied, because of the difficulty of measuring these compounds.

RESULTS AND DISCUSSION

It is evident from the results in Table 1 that the interfacial tension of lipolyzed milk fat was lower than that of the control milk fat. The relatively high ADV of the control samples may be attributed to the age of the 40% cream available at the time. As was anticipated, the removal of FFA in the control samples caused a slight increase in interfacial tension. Contrary to what would be expected, removal of the FFA in the lipolyzed samples resulted in a decrease in interfacial tension. However, the difference between the control and lipolyzed samples was not significant at $P < 0.05$, even though the difference between averages was larger than in the extracted fat. Becher (2) found that, as the concentration of FFA increased with respect to the other surface active compounds present in a system, surface tension decreased to a minimum and then increased.

TABLE 1

Effect of successive removal of free fatty acids, acetone insoluble lipids, and monoglycerides on the interfacial tension of milk fat–water systems

Treatments	Samples [a]		Significance [b]
	Control	Lipolyzed	
Extracted fat			
Acid degree value	2.00	7.55	
Monoglyceride [c]	0.19	1.06	
Interfacial tension [d]	5.4	4.0	0.02
Removal of free fatty acids			
Acid degree value of recovered fat	0.31	0.26	
Interfacial tension	5.9	2.7	N.S.
Removal of acetone insoluble lipids			
Monoglyceride of acetone insoluble lipids	Trace	Trace	
Interfacial tension	11.8	5.4	0.01
Removal of monoglycerides [e]			
Interfacial tension	24.4	22.7	0.01

[a] Average of five trials.
[b] Determined by "t" test. Difference between control and lipolyzed samples. N.S. means nonsignificant.
[c] Total monoglyceride content in m*M*/100 g. fat.
[d] Dynes/cm at 56 ± 1° C.
[e] As part of 80% ethanol soluble lipids.

When the acetone insoluble lipids were removed from both the control and lipolyzed samples, the increase in interfacial tension in the lipolyzed samples was 2.7 dynes per centimeter, as compared to 5.9 in the control samples. It was found upon analysis of the mixed acetone insoluble lipids, which comprised about 1.5% of the extracted fat, that this fraction had approximately 34 mg. of phosphorus per 100 g. of lipid. Since the acetone insoluble lipids contained only a small quantity of phospholipid, the question arose as to which of the lipid constituents removed by acetone precipitation caused the interfacial tensions to rise an average of 5.9 dynes in the case of the control samples and only 2.7 dynes in the lipolyzed samples. The composition of the acetone insoluble lipids as determined by silica-gel column chromatography was triglyceride—85%, diglyceride—12%, monoglycerides—trace, and phospholipid—3%. It, therefore, appeared that the phospholipids and diglycerides influenced the interfacial tension.

The 80% ethanol extraction, which removed the monoglycerides from lipolyzed milk fat, also removed some di- and triglycerides as indicated by the following composition of the 80% ethanol soluble material: 10%—monoglycerides, 70%—diglycerides, and 20%—triglycerides, as determined by silica-gel chromatography. Although it had been assumed that the diglycerides had little effect on interfacial tension (10), the changes in interfacial tension brought about by removal of the 80% ethanol soluble lipids from the milk fat were interpreted as being attributable to both mono- and diglycerides. The influence of diglycerides was suggested in subsequent study, in which the addition of 0.35% of 80% ethanol soluble lipids to fat from which the FFA and monoglycerides had been removed, reduced the interfacial tension from 22.7 to 7.6. In contrast, when milk monoglycerides, concentrated to a purity of 80%, were added at the level of 0.69 m*M* per 100 g. of fat to the heptane fraction, the interfacial tensions were reduced only 3.9 dynes. The reasons for significant differences in interfacial tensions after removal of the monoglycerides may be due to diglycerides or to small amounts of monoglycerides, although analyses for the latter showed only traces.

When the sequence of removal of acetone insoluble lipids was altered, as shown in Table 2, the relative effects on the interfacial tensions were changed from that in Sequence I. The reason or reasons for a lower interfacial tension of the treated extracted fat in the control samples in comparison to the lipolyzed samples are unknown. It could be due to the observed decrease to a minimum, then slight increase and leveling off of interfacial tensions in a system when the concentration of surface active compounds is increased (2). The surface activity of the 80% ethanol soluble lipids was again evident in this phase of the experiment. There was little difference, however, in either the control or lipolyzed samples when the 80% ethanol soluble lipids were removed. Apparently, the relatively smaller amounts of mono- and diglycerides in the control samples were effective in lowering interfacial tension.

The butteroil-water system studied in this investigation is not the same as the milk fat–serum system; therefore, it is difficult to extrapolate the results to

TABLE 2

Effect of successive removal of acetone insoluble lipids, free fatty acids, and monoglycerides on the interfacial tension of milk fat–water systems

Treatments	Samples [a]	
	Control	Lipolyzed
Extracted fat		
Acid degree value	2.09	7.63
Monoglyceride [b]	0.22	1.18
Interfacial tension [c]	2.8	4.9 [d]
Removal of acetone insoluble lipids		
Monoglyceride of acetone insoluble lipids	0.08	0.26
Interfacial tension	4.0	3.2
Removal of free fatty acids		
Acid degree value of recovered fat	0.36	0.38
Interfacial tension	5.1	5.7
Removal of monoglycerides [e]		
Interfacial tension	24.6	23.1

[a] Average of three trials.
[b] Total monoglyceride content in mM/100 g. fat.
[c] Dynes/cm at 56 ± 1° C.
[d] Average of two trials.
[e] As part of 80% ethanol soluble lipids.

those conditions which might be present in natural milk. It was evident that the mono- and perhaps the diglycerides produced in milk fat by lipolysis reduced interfacial tension in the simplified system studied. Also, it seems likely that these surface active compounds would alter to some extent the interfacial tension of fat-serum systems in milk. Other surface and interfacial phenomena in milk also may be affected.

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MILK POWDERS. V. EFFECTS OF THE INTERFACIAL TENSION OF BUTTER OIL IN POWDER ON THE WETTABILITY OF THE POWDER[1]

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SUMMARY

Experiments have shown that a spray-dried, agglomerated milk powder containing approximately 25% low-melting (19-21° C.) butter oil fraction, has about the same wettability and dispersibility at 24° C. as a superior grade of instant skimmilk powder. The interfacial tension (oil-water) of this low-melting butter oil fraction was lower than that of higher-melting fractions which yielded much less wettable milk powders. This suggested that the interfacial tension of the fat may play an important role in wettability.

Milk powders were prepared from homogenized mixtures of butter oil and condensed skimmilk. In one series of experiments surface active agents were added to the butter oil and in another series they were added to the condensed skimmilk. Wettability measurements on the various milk powders showed that the monoglycerides (Myverol) exerted a more pronounced effect on wettability when they were incorporated in the non-fat portion of the milk, and the Span and Tween preparations exerted a more pronounced effect when they were incorporated in the fat portion of the milk. The results showed that alterations in the interfacial tension of the fat component of a milk powder does not of itself exert a marked effect on wettability.

In a previous publication Baker *et al.* (2) reported on the effects of the melting point of the butter fat contained in a milk powder on the wettability and dispersibility of the powder. It was shown that a milk powder containing a low-melting (19-21° C.) butter oil fraction had approximately the same wettability and dispersibility at 24° C. as did a superior grade of instant skimmilk powder. It has also been reported (6) that scum was not present on the milk reconstituted from this powder.

Stone *et al.* (7) concluded from their studies on the self-dispersion of milk powders, in which the butter fat was replaced by corn oil, that the superior dispersibility of the powder was due to the circumstance that the fat was in the liquid state. However, Baker *et al.* (2) have shown that the wettability and dispersibility of powders containing various butter oil fractions did not bear a simple relationship to the circumstance that the fat component was liquid.

Several workers (3-5) have studied the effects of surface active agents on the dispersibility and solubility of milk powders. The present paper deals with the relation between the interfacial tension of the butter fat component of a milk powder and the wettability of the powder.

EXPERIMENTAL PROCEDURE

Interfacial tension of butter oil fractions. Butter oil fractions (MP 19-21, 22-24, 28-30° C.) were prepared by the method described previously (2). The

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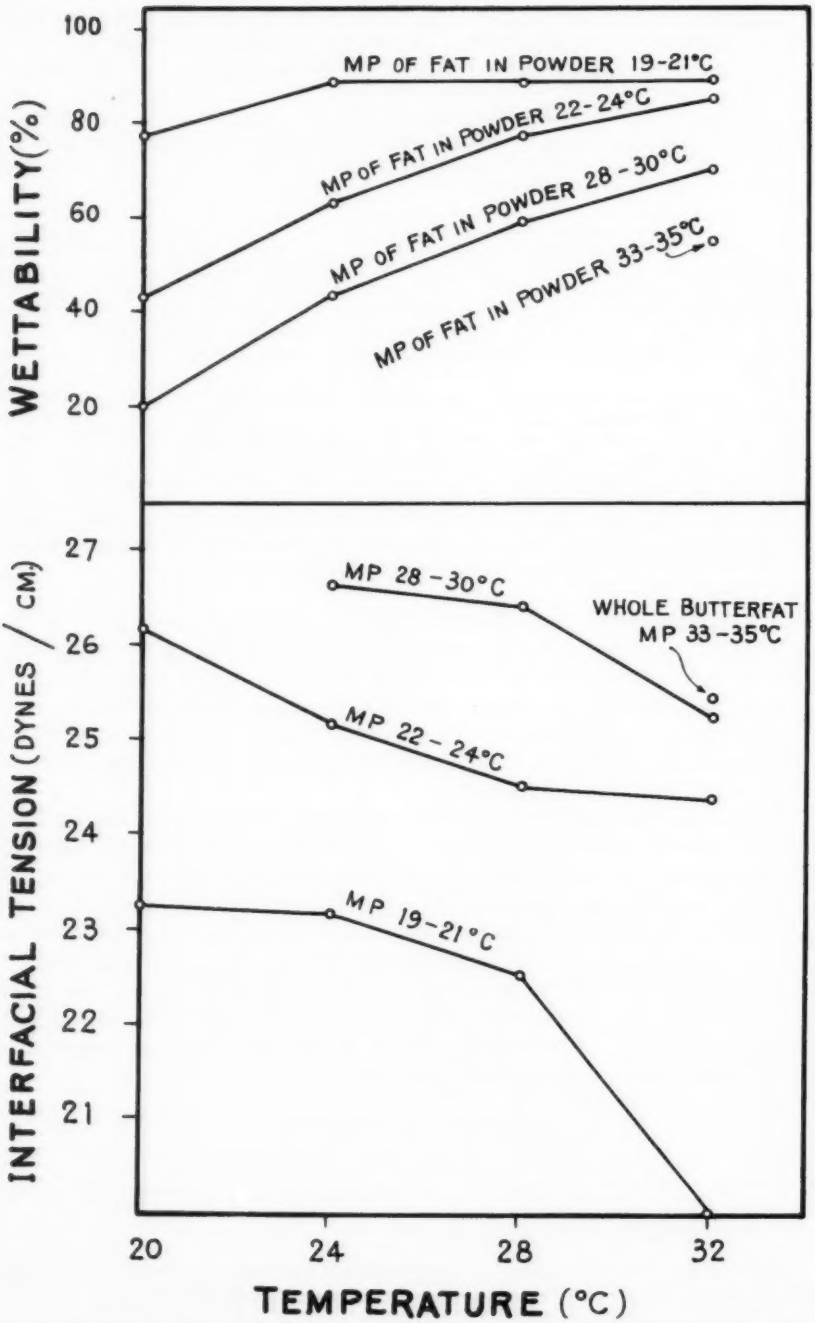

FIG. 1. Effects of temperature on the interfacial tension of butter oil fractions and on the wettability of milk powders containing these fats.

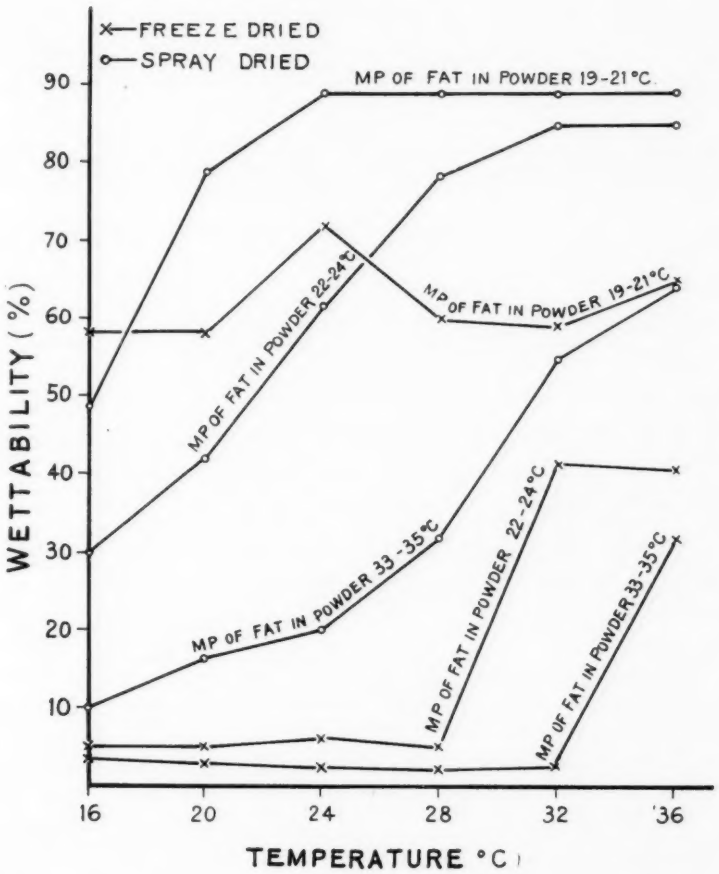

FIG. 2. Effect of temperature on the wettability of spray-dried agglomerated powders and on freeze-dried powders.

interfacial tension (oil-water) of the fat fractions and of whole butter fat was measured by the following procedure. Distilled water (35 ml.) was placed in a 50-ml. beaker and the beaker was adjusted in position on the platform of a DuNouy tensiometer. The platform was raised until the platinum ring was just below the surface of the water in the beaker. Ten milliliters of liquid fat was then added to the beaker and the interfacial tension was measured. When the interfacial tension of supercooled fat was to be determined, the fat was heated to a temperature approximately 5° above its melting point and was then rapidly cooled to the desired temperature and the interfacial tension measurement was made before the fat had solidified. Figure 1 shows the effects of temperature on the interfacial tension of the four fat samples. It also shows

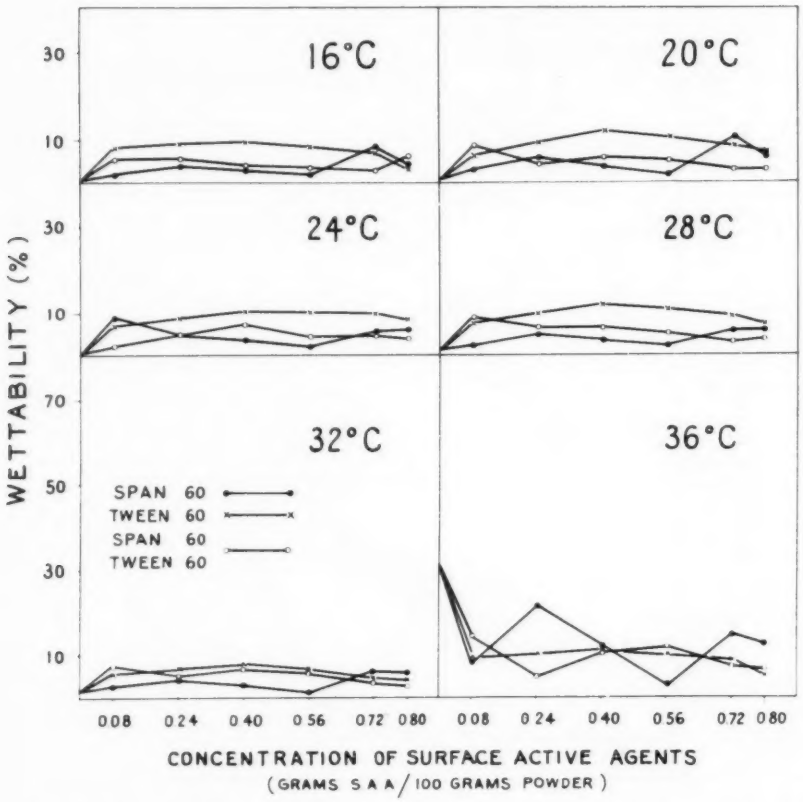

FIG. 3. Effect of addition of surface active agents (Span, Tween) to the nonfat portion of the milk on wettability (Series 1).

the effects of temperature on the wettability (1) of milk powders containing these fats. The powders were prepared by the method described previously (2). Comparison of the wettabilities of any two powders under the same conditions of temperature shows that the powder which has the higher wettability contains the fat of lower interfacial tension.

Effects of surface active agents on the interfacial tension (oil-water) of butter oil. Appropriate quantities of several commercial surface active agents were placed in beakers (150 ml.) and sufficient quantities of butter oil were added to each beaker to give the desired concentration of surface active agent in the fat. The mixtures were stirred at 37° C. for 3 min. and then the interfacial tension was measured by the procedure described above. Table 1 gives the results of this experiment.

Effects of surface active agents on the wettability of whole milk powders. In the following experiments the powders were prepared by freeze-drying. It

TABLE 1

Interfacial tension of butter oil containing surface active agents

	Interfacial tension (dynes/cm) (37° C.)								
Concentration of S.A.A.	Span 60	Tween 60	Span 60 + Tween 60 (50/50)	Myverol (18-00)	Myverol (18-06)	Myverol (18-07)	Myverol (18-40)	Myverol (18-85)	Sample 26706
(%)									
0.00	25.2	25.2	25.2	25.2	25.2	25.2	25.2	25.2	25.2
0.02	18.9	12.5	11.5	19.2	21.1	19.2	21.1	21.1	21.8
0.06	18.7	8.7	6.9	18.4	19.4	19.2	19.6	21.1	21.1
0.10	17.8	6.1	5.9	17.8	18.1	18.3	19.2	20.8	21.1
0.14	16.7	6.1	5.5	16.8	16.3	18.0	19.2	19.4	20.2
0.18	16.0	6.0	5.5	16.0	16.0	17.3	19.2	19.2	19.0
0.20	16.0	5.8	5.5	16.0	15.5	16.0	19.2	19.1	19.0

Span 60 (Sorbitan monostearate), Atlas Powder Company Canada, Ltd., Brantford, Ont.

Tween 60 (Polyoxyethylene sorbitan monostearate), Atlas Powder Company Canada, Ltd., Brantford, Ont.

Myverol 18-00 (Distilled monoglycerides from fully hydrogenated lard), Distillation Products, Rochester, N.Y.

Myverol 18-06 (Distilled monoglycerides from fully hydrogenated vegetable oil), Distillation Products, Rochester, N. Y.

Myverol 18-07 (Distilled monoglycerides from fully hydrogenated cotton seed oil), Distillation Products, Rochester, N. Y.

Myverol 18-40 (Distilled monoglycerides from prime steam lard), Distillation Products, Rochester, N.Y.

Myverol 18-85 (Distilled monoglycerides from refined cotton seed oil), Distillation Products, Rochester, N.Y.

Sample 26706 (Distilled monoglycerides from refined safflower seed oil), Distillation Products, Rochester, N.Y.

was realized that the resulting powders would not be as wettable as powders of the same composition prepared by spray-drying followed by agglomeration. Preliminary experiments were performed to check the differences between the wettability of freeze-dried milk powders and of spray-dried and agglomerated powders of approximately the same composition. The freeze-dried powders were prepared as follows: Twenty-gram lots of butter oil were added to 100-ml. portions of condensed milk (40% solids). The mixtures were heated to 50° C. and then homogenized (Laboratory Homogenizer, ERWEKA, GMbH, Type

TABLE 2

Composition of milk powders prepared by freeze-drying and by spray-drying

Products	Fat *(%)*		Total solids *(%)*	
	Freeze-dried	Spray-dried	Freeze-dried	Spray-dried
Product No. 1 MP of fat 19-21° C.	22.0	24.5	95.2	95.1
Product No. 2 MP of fat 22-24° C.	24.3	24.8	95.0	96.4
Product No. 3 MP of fat 33-35° C.	21.9	25.3	96.8	95.8

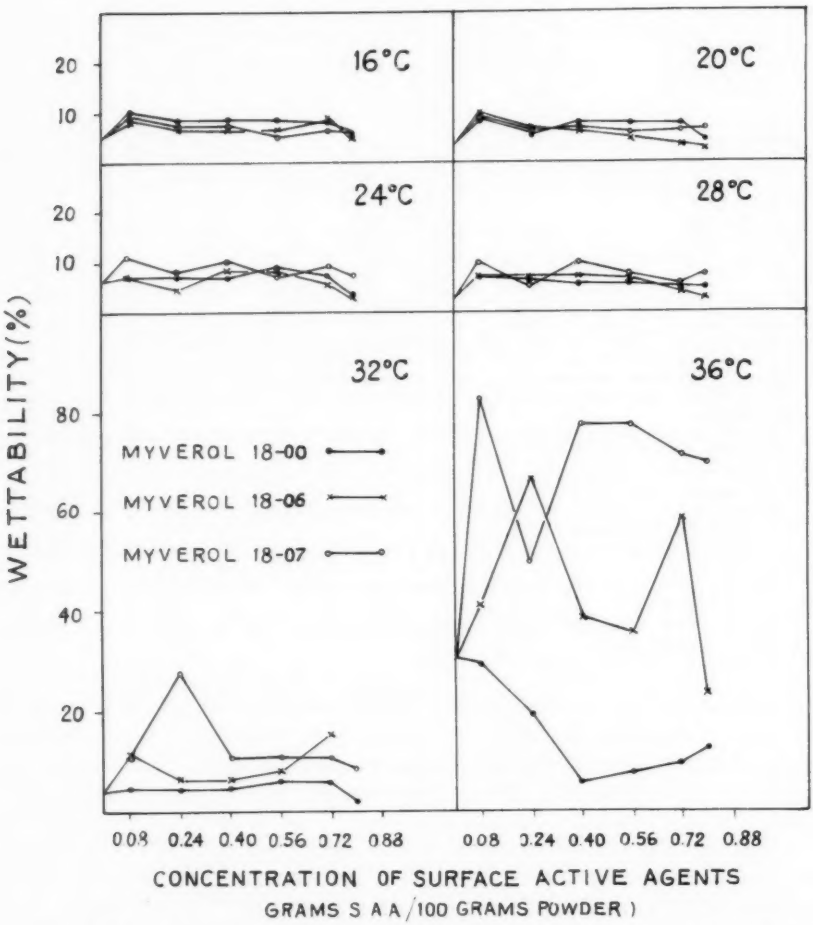

FIG. 4. Effect of addition of surface active agents (Myverol) to the nonfat portion of the milk, on wettability (Series 1).

KUI). The homogenized products were then freeze-dried (Virtis Laboratory Freeze Drier). The dried products were ground in a micro-sample mill (Pulverizing Machinery Co.). The resulting powders were sifted and the fraction which passed through a 1.0-mm. sieve and did not pass through a 0.5-mm. sieve was used for the measurement of wettability (1). Butter oil fractions melting at 19-21, 22-24° C. and whole butter fat melting at 33-35° C. were incorporated in freeze-dried milk powder. The same fats were also incorporated in spray-dried and agglomerated powders by a method reported elsewhere (2). Table 2 gives the results of the analysis of fat and total solids (Mojonnier) of the six products.

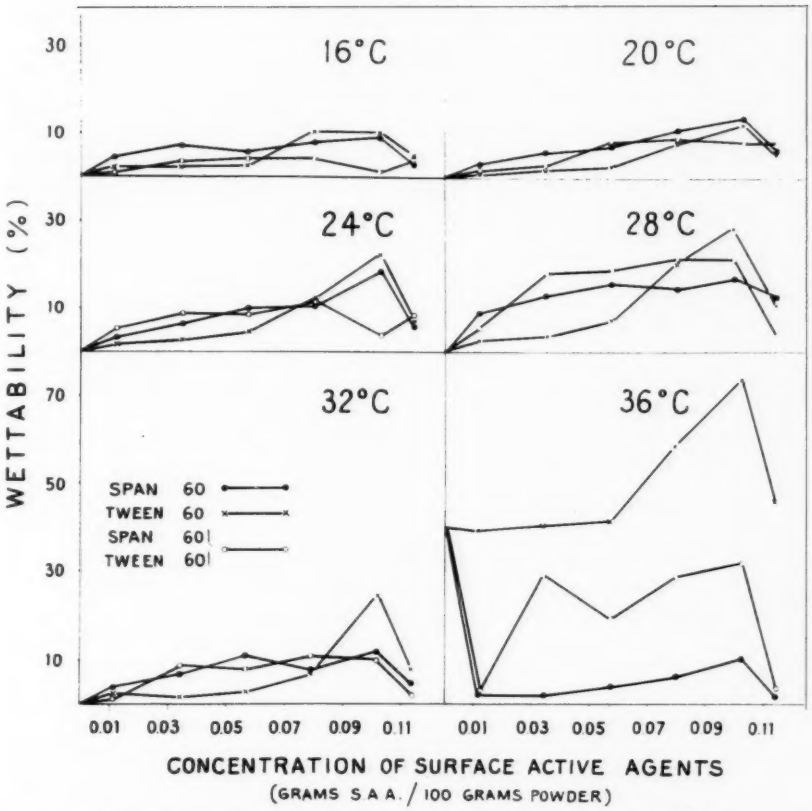

FIG. 5. Effect of addition of surface active agents (Span, Tween) to the fat portion of the milk, on wettability (Series 2).

Figure 2 shows that the wettabilities of the spray-dried and agglomerated powders were higher than those of the freeze-dried powders. It may be noted that the spray-dried powder and the freeze-dried powder with the outstanding wettability characteristics were those that contained the low-melting butter oil.

Two series of experiments have been performed in which the effects of the incorporation of surface active agents in whole milk powders, on the wettability of the powders, have been investigated. In the first series, the surface active agents were added to the condensed skimmilk prior to the addition of the butter fat (unfractionated) and homogenization and freeze-drying of the milk. In the second series, the surface active agents were added to the butter fat (unfractionated). The butter oil was then mixed with condensed skimmilk; the resultant mixture was homogenized and freeze-dried. The freeze-dried products were ground and sifted as previously described. Figure 3 shows the effects of the addition of Span 60, Tween 60, and a mixture (50/50) of Span 60 and

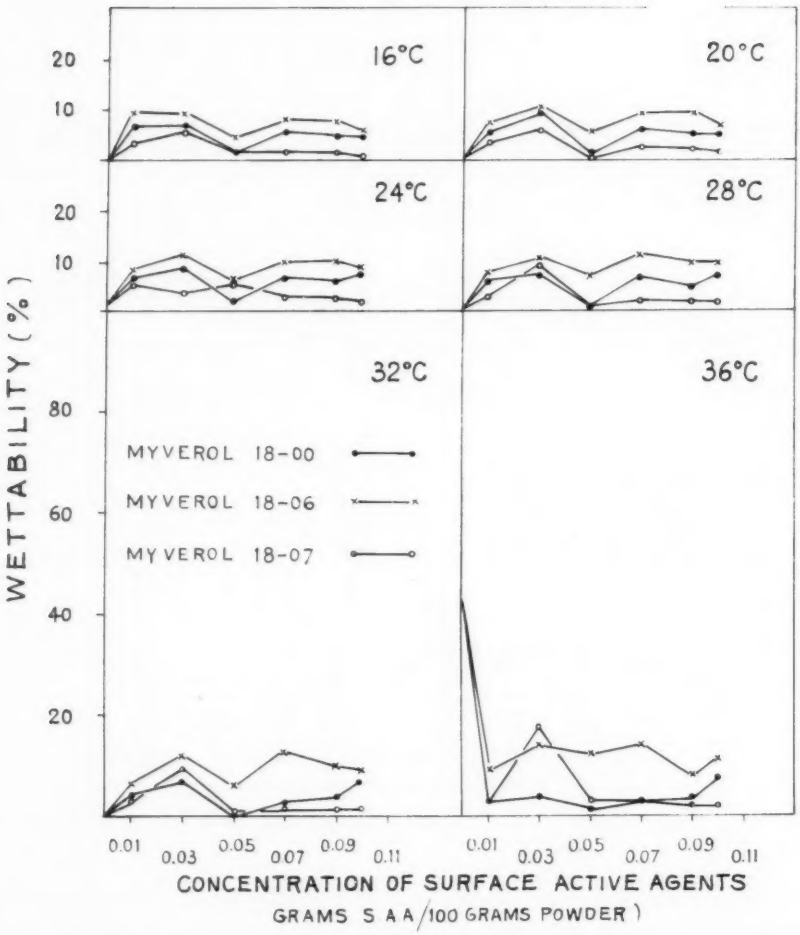

FIG. 6. Effect of addition of surface active agents (Myverol) to the fat portion of the milk, on wettability (Series 2).

Tween 60 (Atlas Powder Company, Ltd., Brantford, Ont.) to the nonfat portion of the milk (Series 1). Figure 4 shows the effect of the addition of Myverol 18-00, Myverol 18-06, and Myverol 18-07 (Distillation Products Industries, Rochester, N.Y.) to the nonfat portion of the milk (Series 1). Figures 5 and 6 show the effect of the addition of the same surface active agent to the fat portion of the milk (Series 2).

DISCUSSION

The addition of the monoglycerides Myverol 18-00, 18-06, and 18-07 to the condensed skimmilk or to the butterfat had little or no effect on the wettability

of the powders at 16, 20, 24, 28, and 32° C. At 36° C. the monoglycerides had an adverse effect on wettability when they were added to the skimmilk. The addition of Myverol 18-00 to the condensed milk had an adverse effect on wettability at 36° C., whereas the addition of Myverol 18-06 and 18-07 tended to improve the wettability.

The addition of Span 60 and Tween 60 to the condensed milk had little effect on wettability at 16, 20, 24, 28, and 32° C. and had an adverse effect on the wettability at 36° C. The addition of Tween 60 to the butterfat improved the wettability of the powder, whereas Span 60 under the same conditions had little or no effect.

These experiences have shown that alteration in the interfacial tension of the fat component of a milk powder does not of itself exert a marked effect on the wettability of the powder if the fat component is not liquid. In the light of the fact that a milk powder containing a butter oil fraction melting at 19-21° C. was very wettable at 24° C., and that interfacial tension of the fat was low, it would seem that the combination of two factors, namely, the physical state (liquid or solid) of the butterfat and interfacial tension of the fat, is important in determining wettability.

ACKNOWLEDGMENTS

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THE DENSITY OF MILK AT LOW TEMPERATURES [1]

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SUMMARY

The densities of a variety of herd milks from representative sources in the Washington, D. C., area were determined at low temperatures, to establish volume-weight relationships more accurately than was possible from existing data.

Density measurements were made in duplicate over a range of 1 to 10°C. on 101 samples of milk. Specially designed pycnometers, having a capacity of about 46 ml., were used in refrigerated water baths controlled to within ± 0.02° C. The percentage of milk fat in most of the samples was determined by the Babcock method, and in the remainder by the Mojonnier method. The percentage of total solids in 85 samples was determined gravimetrically.

The percentages of fat, total solids, and nonfat solids covered the following ranges, respectively: 3.1 to 6.4; 11.6 to 16.1; and 8.2 to 10.2. The range of densities at 0.95, 4.95, and 9.85°C. were, respectively: 1.0312 to 1.0386; 1.0322 to 1.0373; and 1.0298 to 1.0368.

Formulas for calculating the density of milk were derived from the data by the method of least squares. The results emphasize that density is more closely correlated with the percentage of nonfat solids than with the percentage of fat in milk.

Information on the density of milk having different percentages of fat for temperatures of practical interest has been available for many years. Values in tabular form (3) are used generally by milk processors for calculating the payments for milk, and for estimating weight yields of dairy products from the volume in gallons and the percentage of fat by weight. The tables are based on a study of the density of milk which was made by Bearce (2) at the National Bureau of Standards about 45 yr. ago on a relatively few samples. Several values at low temperatures were not used, because they could not be satisfactorily included in the formulation of a simple equation for densities between 20 and 50° C. Some doubt concerning the accuracy of the data was expressed in the report. This was attributed to some separation of fat in the samples during the experiments, and to the fact that the values for densities at low temperatures were obtained by extrapolation from the determinations made at 20° C. and higher. The suggestion was made that further work should be done using a method for determining density which would be more adapted to the nature of milk.

Published information concerning the density of milk at low temperatures is rather meager. Wegener (15) reported values for pasteurized milk in the range

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of 10 to 75° C., but did not include the effect of milk composition. Thomsen (14) constructed a table for calculating the weight of milk per gallon over the range of 36 to 160° F. (2.2 to 71.1° C.) which is based on a combination of the early data from the Bureau of Standards and those of Jacobson (5). He verified experimentally a random number of the values. Short (13) determined the density of raw milk between 10 and 45° C., and reported the variation of the temperature coefficient of density for milks of different composition. Recently, Whitnah *et al.* (16) investigated the density of milk and found a smooth curve for density vs. temperature near 4° C. The average temperature of maximum density was −5.20° C.

Reliable density values are especially needed in the low temperature range, since milk is now largely stored in farm tanks and transported in tank trucks at temperatures near 40° F. (4.4° C.). Therefore, this investigation of the density of milk over the range of 1 to 10° C. was made in order to fill the void in the existing information, and to obtain data which are necessary for the preparation of density tables for practical use. Tables which give the density of milk at low temperatures in pounds per gallon for different percentages of fat and nonfat solids will be presented in another publication.

EQUIPMENT AND MATERIALS

Pycnometers. Densities were measured by the pycnometer method, using specially designed pycnometers (Figure 1). A special feature is an upper cup which holds an excess of the sample above the orifices of the capillaries, thus permitting an automatic adjustment of the milk volume when temperature equilibrium is reached. The pycnometers were calibrated with double-distilled water at 0.95, 4.95, and 9.85° C.

Constant temperature baths. Stainless steel water baths equipped with electric stirrers and certified mercury thermometers, and containing distilled water, were controlled to within ± 0.02° C. A Beckman differential thermometer served in estimating the readings to 0.01°. The densities were determined in a room which was held at a constant temperature of 20° C.

Milk samples. A total of 101 samples of mixed raw milk representing all months of the year were tested. Seventy-four were obtained from the U. S. Department of Agriculture herd at Beltsville, Maryland. These samples were mixed morning milk from four to six cows of the Holstein, Jersey breeds and cross-breeds of Holstein-Sindhi and Holstein-Jersey-Red Dane. The samples were cooled promptly to about 40° F. and 60 of them were held for from 4 to 6 hr. and 14 of them for about 24 hr. prior to starting the density determinations.

Eleven samples were obtained from the University of Maryland dairy, nine from the University herd, and two from nearby farms. The University herd milk was mixed morning and evening milk from a herd of 75 cows, which comprised Ayrshire, Holstein, Jersey, and Guernsey breeds. Three samples represented milk held for about 17 hr. and the remainder for about 4 hr. at about 40° F.

Ten samples supplied by the laboratory of the District of Columbia Department of Health came from milk shipped from Maryland and Virginia farms

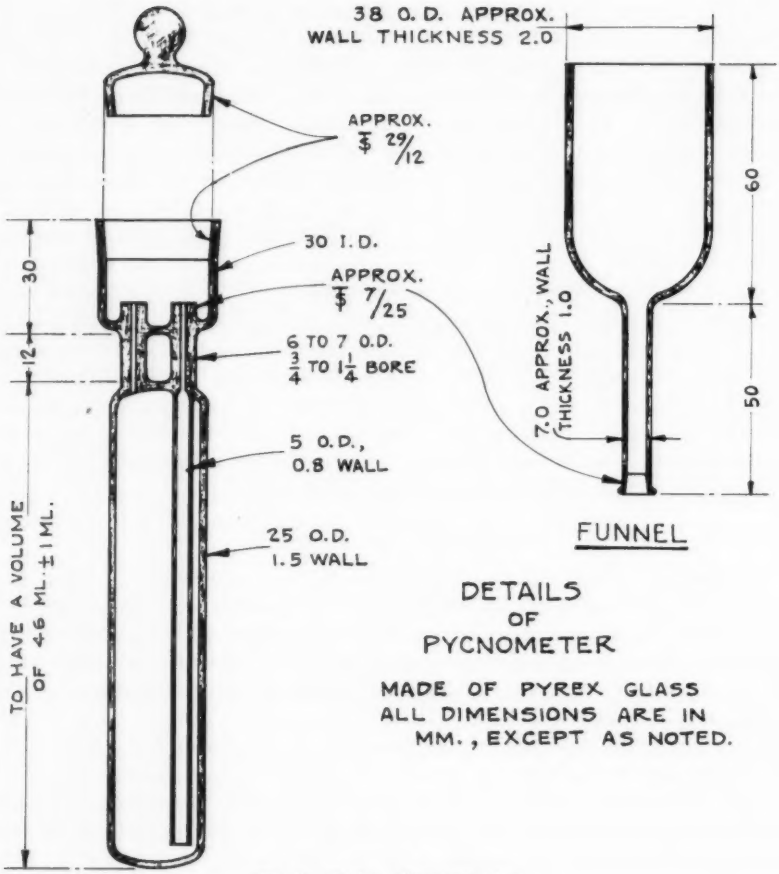

FIG. 1. Design of pycnometer.

within a radius of about 40 miles. The samples represented from 2,800 to 32,000 lb. of farm tank or tank truck milk from two or more producers. These samples represented commercial fluid milk. It may be presumed that they consisted largely of Holstein milk, since it has been reported that approximately 85% of the cows in the District of Columbia milk shed are Holsteins (18). Information was not available for the tank-holding period of this milk.

Six samples of Guernsey milk supplied by two District of Columbia dairies were from tank trucks which contained from about 4,300 to 8,400 lb. of milk. Four samples were from milk held about 24 hr. and two samples from milk held about 2 hr. at 40° F.

EXPERIMENTAL METHODS

Density determinations. Simultaneous density determinations were made in duplicate at two temperatures for each sample of milk: 54 were measured at

0.95 and 4.95° and 47 at 0.95 and 9.85° C. The occlusion of small air bubbles in the pycnometers was avoided by slowly pouring the first portion of milk into the funnel while the pycnometer was in a slanted position. The milk was allowed to overflow from the other capillary, and the level of milk in the cup was adjusted so that the orifices were immersed to a depth of about 2 mm. The cap was placed on the cup and the pycnometer immersed in the bath to the level of the milk in the cup. The design of the pycnometer facilitated rapid chilling of the milk; thus, the volume of milk rapidly attained a state of equilibrium. This arrangement and the gentle agitation of the milk in the cup caused by vibrations of the cooling equipment and stirrers tended to reduce any errors which might be due to the rising of milk fat in the cup during contractions of the milk.

The filled pycnometers were held in the baths for a period of about 1 to 1.5 hr. for the density determinations. Subsidiary experiments indicated that fat in milk which had been previously cooled to about 40° F., and then held for about 1 hr. in the refrigerated baths, was in a state of physical equilibrium; therefore, the milk fat was largely solidified, and its density was at a maximum during the determinations.

After temperature equilibration, each pycnometer was raised slightly in the water baths, the cap removed briefly, and most of the excess milk in the cup withdrawn with a small rubber-tipped suction tube. The surface of the cup was cleaned with small pieces of lintless tissue which had been moistened with distilled water–acetone solution (about 70:30) and wrapped around small bevel-ended wooden sticks, and finally with dry tissue.

All weighings of the pycnometers were made at 20° C. with an analytical balance. The weights were calibrated by comparison with standard weights, certified by the National Bureau of Standards. A correction for the air buoyancy was applied to the apparent densities. A tare pycnometer eliminated any correction for the vessel itself, and the effects of variations in the humidity and temperature.

Determinations of milk fat and total solids. Gravimetric determinations of the percentage of fat were made on the first 16 samples by the Mojonnier method (9), and the Babcock method (1, 8) was employed for the remainder, on which solids also were determined. Accurately calibrated Babcock bottles and pipettes, certified by the Dairy Inspection Service of the State of Maryland, were used. The fat readings were estimated to 0.05 per cent by means of an illuminated reader with a magnifier. The percentage of total solids was determined by the Mojonnier vacuum-oven method (9). The determinations were carried out in duplicate.

RESULTS

The data before analysis are not presented here because of their number. Photostats of the tabulated results can be obtained by writing to the author.

The relationships between density and percentages of nonfat solids, fat, and total solids at 0.95° C. are shown in Figures 2, 3, and 4, respectively. Only the results obtained at 0.95° are presented in detail because all of the samples were tested at this temperature; whereas, only about half of them were tested at 4.95°

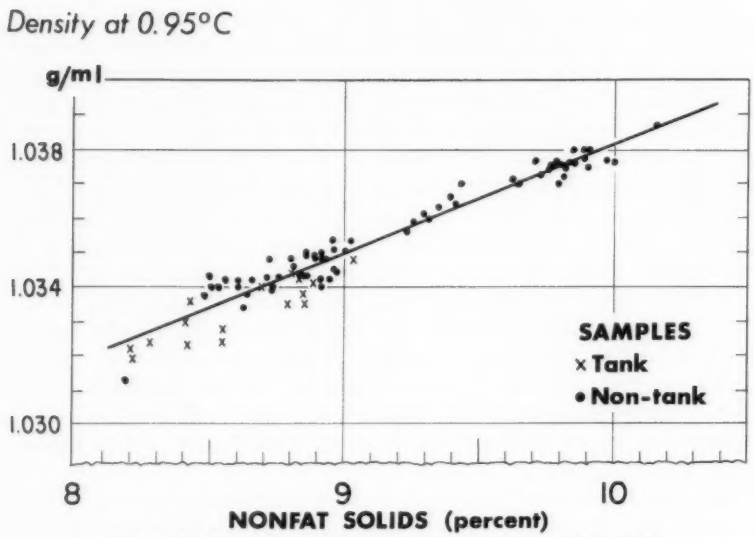

FIG. 2. Relationship of density to percentage of nonfat solids.

and the other half of them at 9.85° C. Furthermore, in general, the results at 4.95 and 9.85 were similar to those obtained at 0.95° C., except for a slight difference due to temperature.

It should be noted that the density values in relation to nonfat solids (Figure 2) are much closer to the line than those in relation to either fat or total

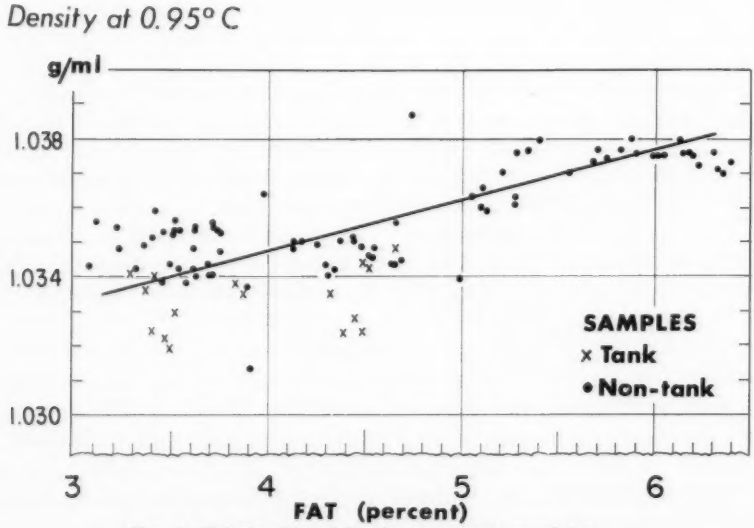

FIG. 3. Relationship of density to percentage of fat.

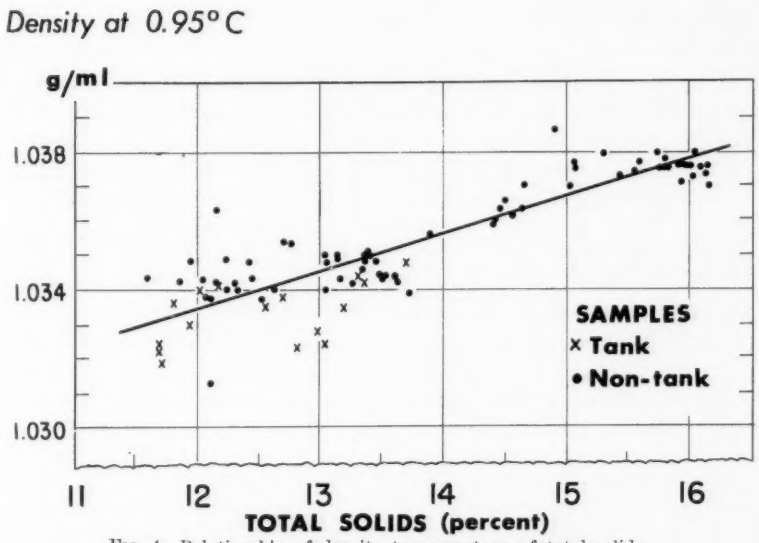

FIG. 4. Relationship of density to percentage of total solids.

solids (Figures 3 and 4). Also, the density was affected as much by a 20% difference in nonfat solids content as by about a 123% difference in fat or by about a 44% difference in total solids over the range of these measurements.

A number of the tank truck samples gave anomalously low values for density and total solids, perhaps indicating the presence of extraneous water.

STATISTICAL ANALYSIS

On the basis that density can be expressed as a linear function of temperature and percentages of fat and nonfat solids over a short range, the best fitting function can be determined by the method of least squares.

If only the percentage of nonfat solids is considered, the function is:

$$D = 1.006830 - .000179\,T + .003144\,N \qquad (1)$$

Where D = density in gram/milliliter

T = temperature in degrees C.

N = percentage of nonfat solids in the milk.

The 95% confidence limits on densities estimated by this formula are D ± .000875. These narrow confidence limits result from a 2.25-fold reduction in the standard error.

When only the percentage of fat is considered, the similar function is:

$$D = 1.028863 - .000195\,T + .001432\,F \qquad (2)$$

Where F = percentage of fat in the milk.

The 95% confidence limits on an estimate of density by this function, used as a formula within the range under investigation, are approximately D ± .00198.

If the percentages of both fat and nonfat are considered, the best fitting function is:

$$D = 1.003073 - .000179\,T - .000368\,F + .003744\,N \qquad (3)$$

The 95% confidence limits on densities estimated by this formula are $D \pm .000812$.

It may be observed that there is but slight advantage in the use of the percentage of fat in addition to that of nonfat solids.

If nonfat solids and fat are considered together as total solids (S), then the corresponding fitted function becomes:

$$D = 1.021110 - .000188\,T + .001046\,S \qquad (4)$$

The 95% confidence limits on densities estimated by this formula are approximately $D \pm .00154$. It may be observed that this function does not provide an appreciably better estimate than the function which considers fat only.

Comparison of results with published values. Values obtained by the extrapolation of Bearce's data (2) below 20° C., as he suggested, were found to be about 0.3 to 0.4% higher than the densities for 3.5% fat milk calculated by Formula (2) for temperatures of 1, 5, and 10° C. His observed densities for 2.5, 3.5, and 5% fat milks averaged about 0.2% higher over the temperature range of 0 to 10° than densities calculated by Formula (2). Bearce reported that the densities were in most cases somewhat too large.

The density of raw milk containing 4% fat and 9% nonfat solids calculated for 10° C. by the formula of Short (13) was about 0.13% lower than the density calculated by Formula (3).

The average densities of pasteurized, and pasteurized and homogenized milks containing 4% fat and 9% nonfat solids reported by Rutz *et al.* (12) for the temperature range of 0 to 8° C. were about 0.05% lower than densities calculated by Formula (3). Whitnah *et al.* (16) published density values for pasteurized, and pasteurized and homogenized milks containing 3.6 fat and 9.1% nonfat solids at the temperatures of 3 and 5° which were 0.15 to 0.18% lower than densities calculated by Formula (3).

Density values expressed in pounds per gallon in Thomsen's table (14), for 3, 4, and 5% fat milks at temperatures of 40 and 50° F., are almost identical with densities calculated by Formula (2) and converted into apparent weights in pounds per gallon at these temperatures.

The agreement between the density values for milk from various sources is noteworthy, when the variations in milk and its treatment, and the different methods used in determining density, are considered. This is especially true because of unavoidable variations in milk and in sampling it. Rutz *et al.* (12) found that the standard deviations for the density of milk from one herd of cows were large when compared with the estimated precision of measurement. It should be expected that such deviations would be even greater when samples of milk are obtained from various sources and under various conditions of sampling.

DISCUSSION

The literature shows that the density of nonfat solids in milk at 10° C. is approximately 1.6 times that of water, whereas the density of milk fat is 0.93 times that of water. Thus, the density of nonfat solids is 0.6 greater than water, and that of fat is only 0.07 less than that of water. Also, there is usually slightly more than twice as much nonfat solids as fat in whole milk. Thus, the nonfat solids are the most important constituents affecting the density of milk, and this is evident from the results obtained in this study, which show that density is more definitely correlated with the percentage of nonfat solids than with the percentage of either fat or total solids (Figures 2, 3, 4). Obviously, any table involving the volume and weight relationship of milk would have a higher degree of accuracy if the nonfat solids of milk, as well as the fat, were taken into consideration in its preparation and use.

The composition and, hence, the density of the nonfat solids is correlated with the percentage of fat in milk. The density and the percentages of protein and ash increase, but the percentage of lactose decreases, with an increase in the percentage of fat in milk. The ratio of lactose to protein increases about 46% as the percentage of fat decreases from 6.5 to 3.0 (10). Recent studies have indicated that the density of the nonfat solids decreases about 0.02 as the percentage of fat in milk increases from 3 to 6 (17).

The density of milk fat also varies with its composition. The differences between the average maximum and minimum values for the density of milk fat, reported from several sources, varied between about 0.003 and 0.004 (6, 7, 11).

Temperature and its effect upon the physical state of the fat, and particularly the variations which occur in the ratio of the fat and the nonfat solids of milk, are major factors influencing density. Milk fat contracts slightly upon solidification when milk is cooled, and differences in specific gravity may be as great as 0.0002 for each per cent of fat in the milk, depending upon its temperature history (4).

ACKNOWLEDGMENTS

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ROLE OF XANTHINE OXIDASE IN THE REDUCTION OF RESAZURIN BY RAW MILK[1]

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SUMMARY

The addition to raw milk of a variety of substrates for xanthine oxidase resulted in an almost immediate reduction of resazurin. When no substrate was added, doubling the concentration of xanthine oxidase in raw milk by the addition of purified enzyme did not cause a significant increase in the rate of resazurin reduction, confirming the conclusion that the unavailability of substrate limits the reducing activity of this enzyme. Milk from two cows which were very low in xanthine oxidase did show an increased reduction rate on the addition of the purified enzyme, but the final rate was still much too slow to have any bearing on the commercial grade of the milk. The pasteurization of milk destroyed the ability to reduce resazurin but had little influence on the activity of xanthine oxidase. The addition to milk of folic acid, a very effective competitive inhibitor for xanthine oxidase, failed to slow the rate of resazurin reduction significantly. All of these factors point to the fact that xanthine oxidase does not play a significant role in the reduction of resazurin by raw milk.

Although the rate of reduction of resazurin by a sample of raw milk, in general, is an excellent measure of the bacterial content of the milk (5) and a useful aid in detecting excessive numbers of leucocytes (3), nonetheless there are other agents in milk which may contribute significantly to the reduction process (2). These additional agents can apparently cause the rapid reduction of resazurin in the absence of excessive numbers of bacteria or leucocytes. Since Schardinger's pioneer work (9), milk has been cited as an excellent source of xanthine oxidase, an enzyme capable of reducing dyes such as methylene blue or resazurin. This enzyme is considered to be associated with the fat layer of milk and in particular with the lipoproteins of microsomes (7). Campbell and Phelps (2) found that the rapid rate of resazurin reduction, associated with milks of high leucocyte content, was not due to the leucocytes themselves but to some accompanying factor. Nilsson (8) has shown that mastitic milk, of high leucocyte content, was remarkably high in xanthine oxidase and in such milk the amount of enzyme was not a function of the fat content as was true of normal milk.

The present work was undertaken in an effort to determine whether xanthine oxidase contributes significantly to the reduction of resazurin in raw milk of low bacterial content.

MATERIALS AND METHODS

Evening milk was collected immediately after milking and was stored overnight at 4° C. before being used, unless otherwise mentioned. The direct micro-

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scope count for leucocytes and the Standard Methods Plate Count were performed routinely. The resazurin test was carried out by adding 5 ml. of milk to 0.5 ml. of standard resazurin solution in 14-mm. test tubes. The tubes were inverted three times and incubated in a water bath in the dark at 37° C. The milks were then mixed at hourly intervals by inverting the tubes. The Munsell 7/4 standard was taken as the end point. When the end point was reached at from 1½ to 2 hr., there was an error between replicates of ± 10 min., at 3 hr. the error was ± 20 min., and the error increased with increasing reduction time.

Xanthine oxidase was assayed by determining the rate of conversion of hypoxanthine to uric acid (10). The rate of formation of uric acid was followed for a 10-min. period at 290 mμ in the Beckman DU spectrophotometer. A unit of activity is defined as that activity causing an increase in absorbance of 0.001 per minute at 25° C., pH 7.5.

Xanthine oxidase was prepared from raw cream (4). Purification of the enzyme was pursued only as far as elution from aluminum hydroxide gel. Glutathione was added to the assay mixture in order to ensure maximum activity, and under these conditions the preparation contained 150,000 units of enzyme per milliliter.

EXPERIMENTAL AND DISCUSSION

Although milk is known to contain large quantities of xanthine oxidase, one would conclude from Schardinger's experiments, in which he showed that the addition of formaldehyde to milk caused a rapid reduction of methylene blue, that it is deficient in substrate for this very nonspecific enzyme. To confirm this suggestion, a variety of known substrates (1) were added to milk containing resazurin and the reduction rates determined (Table 1). It is evident that if

TABLE 1

Influence of adding substrates for xanthine oxidase on the rate of reduction of resazurin in raw milk

Substrate	Reduction time *(min.)*
Water	180
Hypoxanthine	1
Formaldehyde	2
Adenine	7
Inosine	8
Vanillin	7
Aldol	2

substrates were available in milk, there is sufficient xanthine oxidase present to reduce resazurin in a matter of minutes.

Another approach to this question was to increase the xanthine oxidase of milk by the addition of purified enzyme and observe the effect on the rate of resazurin reduction (Table 2).

It is apparent from these data that there is no obvious correlation between the amount of xanthine oxidase in the milk and the rate of resazurin reduction. This observation has been confirmed, using a much larger group of animals.

TABLE 2

Influence of increasing the xanthine oxidase content of raw milk on the rate of reduction of resazurin

Cow No.	Xanthine oxidase *(units/ml)*		Reduction time *(min.)*	
	Milk	Milk plus xanthine oxidase	Milk	Milk plus xanthine oxidase
1	396	696	125	110
2	366	666	335	305
3	342	642	305	280
4	290	590	65	55
5	280	582	160	145
6	252	553	80	65
7	210	510	140	135
8	120	215	535	435
9	84	185	255	215

The other conclusion is that, in general, doubling the xanthine oxidase content has little, if any, effect on reduction time for, with the exception of milk from cows numbered 8 and 9, the change is within the range of experimental error. However, milk from these last two cows consistently was low in xanthine oxidase and responded to the addition of this enzyme. The addition of higher concentrations of enzyme failed to further increase the rate of resazurin reduction. It would appear that at least in these two milks, which always had a very slow rate of resazurin reduction, the limiting factor in reduction of resazurin was the concentration of xanthine oxidase. None of the milks used in this experiment had greater than 13,000 bacteria or 500,000 leucocytes per milliliter.

Xanthine oxidase is known to be a very heat-stable enzyme and advantage is taken of this fact in its purification. In spite of this, heating milk destroys most of its ability to reduce resazurin, as shown in Table 3. It can be seen that

TABLE 3

Influence of pasteurization on xanthine oxidase content and resazurin reduction time of milk

	Xanthine oxidase *(units/ml)*		Reduction time *(min.)*	
	Raw	Pasteurized	Raw	Pasteurized
Cow No. 1	528	564	240	>510
Cow No. 2	300	258	90	>510
Cow No. 3	258	228	105	>510

the xanthine oxidase content of the milks was not significantly decreased by pasteurization, although the ability to reduce resazurin was lost. These data again point to the minor role that this enzyme plays in the reduction test, even when other agents like bacteria and leucocytes have been severely restricted.

Although the data presented indicate that the concentration of xanthine oxidase in milk is normally not the determining factor in the rate of resazurin reduction, it is still possible that the rate at which substrates become available to this enzyme could be the rate-limiting factor. That is, the substrate or precursors for substrates, such as nucleic acids and the enzymes which convert the precursors to substrates, could be limiting and could be destroyed by pasteurization. The addition of ribonuclease and desoxyribonuclease to milk, however,

did not increase the rate of reduction. The addition of a competitive inhibitor which would selectively inhibit xanthine oxidase would allow a determination of the role of all of the systems which contribute to the action of xanthine oxidase in milk. Fortunately, Kalekar and Klenow (6) have shown that such an inhibitor is present in commercial preparations of folic acid.

In preliminary experiments designed to determine the effectiveness of the inhibitor folic acid, at a final concentration of 0.63 mg. %, was added to the reaction mixture in the xanthine oxidase assay and inhibition was measured by the decrease in uric acid production.

Under these conditions, the xanthine oxidase activity of the purified enzyme or of milk was almost completely inhibited. However, this was when only one substrate, namely hypoxanthine, was used. It is possible that in milk, the normal substrate is an aldehyde and perhaps has a different site of attachment on the enzyme. To protect against such a possibility, a variety of substrates was used (Table 4). Two things may be concluded from these data: The first is that the

TABLE 4

Inhibition of xanthine oxidase activity by folic acid as evidenced by decreased resazurin reduction rate

Substrate	Reduction time *(min.)*	
	Control	Folic acid added
Water	105	115
Hypoxanthine	1	75
Formaldehyde	2	80
Adenine	7	115
Inosine	3	105
Vanillin	7	105
Aldol	3	75

addition of folic acid to milk had little, if any, influence on the rate of reduction. Secondly, the commercial folic acid is a very efficient inhibitor of xanthine oxidase, irrespective of which substrate is used.

If the xanthine oxidase system is active in the reduction of resazurin in milk, the addition of folic acid should increase the reduction time. The data of the previous table were extended by determining this effect on the milk from seven additional cows (Table 5). Again, it would appear that xanthine oxidase does not play a significant role in the resazurin reduction test.

TABLE 5

Influence of folic acid on rate of resazurin reduction by raw milk

Cow No.	Reduction time *(min.)*	
	Control	Folic acid added
1	350	355
2	70	70
3	260	275
4	105	115
5	180	190
6	80	80
7	95	100

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VARIATIONS IN THE HEAT STABILITY AND COMPOSITION OF MILK FROM INDIVIDUAL COWS DURING LACTATION[1]

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SUMMARY

Milk samples from each of two cows were collected at intervals throughout a lactation period and analyzed for heat stability (over a range of pH), various inorganic constituents, and seven protein constituents (by electrophoresis).

With all except a few early-lactation samples, a sharp maximum in heat stability could be demonstrated by adjusting the pH over a narrow range, usually between 6.6 and 6.7. The heat stability of the untreated milk appeared to be determined by the change in acidity (H^+ concentration) required to adjust the milk to the pH of maximum heat stability.

When the data for both cows are considered, the heat stability of the original milk was not correlated with any constituent(s) of the milk. The maximum heat stability, on the other hand, was significantly correlated with the ratios: Calcium Ion/Soluble Inorganic Phosphorus, Soluble Calcium/Soluble Inorganic Phosphorus, and Soluble Calcium plus Soluble Magnesium/Soluble Inorganic Phosphorus plus Soluble Citrate.

Results of various workers have shown that although the heat stability of milk responds to trace additions of various salts it does not correlate with any known constituent of the milk, or with any combination of such constituents (7, 10, 23, 29, 34). It has also been shown that samples made by mixing milk from two individual cows do not behave in a predictable manner (4). Possibly, heat stability can vary with a multiplicity of factors, some of which may be ineffective in some milks. Bulk herd milks tend to achieve an average chemical composition, but they must also tend towards a maximum complexity so that no one factor exerts a detectable control over the heat stability. Milk from individual cows, on the other hand, varies considerably in composition (21, 29) and in heat stability (12,26) from day to day, but its complexity may actually be less than that of bulk milks.

A recent intensive attempt to correlate the heat stability of milk with its chemical composition (33,34) includes data on samples from individual cows at different stages of lactation, but the reports do not identify the samples from any one cow throughout a lactation period. Much of the published data that do permit this comparison cover only short periods (26) or lack the heat stability or other data (11, 12, 14, 21, 29). Further studies on the composition and heat stability of milk from individual cows were, therefore, undertaken. The results presented herewith extend earlier data on the composition of milk by including an electrophoretic analysis of centrifugally separated casein and serum at each sampling date.

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MATERIALS AND METHODS

Milk samples. Milk from two Holstein cows was used in this study. Cow No. 1 (Ottawa Commander Aggie) freshened on October 25, 1958, and dried off August 9, 1959, having produced 12,202 lb. of milk during the 287-day period. She was in good health except for a mastitis infection treated successfully (with neospan and penicillin) on the 50th day post-partum. She was barn-fed on hay, corn, and meal until the 212th day, then pasture-fed on legume and grass plus a meal supplement throughout the rest of the lactation period, except for 1 wk. (223rd to 228th day, inclusive) on oat pasture.

Cow No. 2 (Ottawa Batty Virginia) freshened for her first lactation period on September 21, 1959, and dried off on July 16, 1960, having produced 7,604 lb. of milk during the 300-day period. Throughout this period she was in good health, and was barn-fed on hay, silage, and meal.

All samples were taken from morning milkings and defatted by centrifugation.

Methods. Raw skimmilk and ultrafiltrate, prepared as previously described (30), were analyzed as summarized in Table 1. The pH was determined at room temperature on a Beckman Model G apparatus with a general purpose electrode.

Heat stability was determined as previously described (26) on the untreated skimmilk, and on series of samples in which the composition had been altered by small additions of either hydrochloric acid (0.1 *N*), sodium hydroxide (0.1 *N*), calcium chloride (0.1 *M*), or sodium phosphate (0.1 *M* mixture of mono- and

TABLE 1
Analytical methods

Component	Fraction	Solution analyzed	Method
Calcium	Total	Skimmilk [a]	Oleate (16)
	Total soluble	Ultrafiltrate	Oleate (16)
	Ionic	Ultrafiltrate	Murexide (30)
Magnesium [b]	Total	Acid serum of skimmilk	Erucicate (18)
	Soluble	Ultrafiltrate	Erucicate (18)
Phosphorus	Total	Wet ash (1) of skimmilk	Phosphomolybdate (22)
	Total soluble [b]	Wet ash (1) of ultrafiltrate	
	Total inorganic	T.C.A. filtrate of skimmilk	
	Soluble inorganic	Ultrafiltrate	
Citrate	Total	Skimmilk	Acetic anhydride-pyridine (17, 19)
	Soluble	Ultrafiltrate	
Nitrogen	As per Rowland's procedure (27)		Micro-Kjeldahl, sodium sulfate–mercuric oxide catalyst

[a] With late-lactation milks, total calcium was determined in acid serum because of casein instability.
[b] Cow No. 2 only.

di-phosphate, pH 6.7). The maximum addition was 1.2 ml/20 ml. sample, and tests showed that even this degree of dilution did not affect the shape of the curve obtained by plotting the additive vs. heat stability. Dilution of the central samples of each series was, therefore, kept to a minimum by not adding water to adjust the whole series to an equal volume. All samples were equilibrated overnight before determining the heat stability and pH.

For electrophoresis, casein and serum were separated by centrifugation at approximately 80,000 × g (Spinco Model L, No. 30 rotor, 30,000 r.p.m.) for 1 hr. The sedimented casein was redispersed in veronal buffer (pH 8.3, ionic strength 0.1), and both the redispersed casein and the serum were dialyzed at 4° C. for 48 hr. against two changes, of more than two liters each, of this buffer. After dialysis, the solutions were clarified by recentrifugation under the same conditions for 30 min.; if necessary, clear serum was withdrawn from beneath the cloudy lipid-containing layer. Electrophoresis was then carried out in a standard 11-ml. cell on a Spinco Model H apparatus, at 1° C., for 300 to 400 min. at 10 ma. (approximately 78 V).

TABLE 2

Concentration of minor protein constituents
(μg. N/ml milk)

Component	Cow No.	Early lactation (4th day)	Mid-lactation		Late lactation [a]
			Range	Average	
γ-casein	1	0.24	0.11–0.58	0.26	0.71
	2	0.37	0.17–0.24	0.20	1.48
Immune globulins	1	0.36	0.06–0.14 [b]	0.11 [b]	0.61
(Peaks 1 and 2)	2	0.80	0.08–0.20	0.12	0.89
Blood serum albumin	1	0.07	0.02–0.10	0.04	0.16
(Peak 7)	2	0.09	0.03–0.08	0.05	0.52

[a] Two hundred and eighty-third of 287 days for Cow No. 1; 295th of 300 days for Cow No. 2.

[b] Excluding high values associated with mastitis infection.

The relative area under each of the electrophoretic peaks was determined for the descending boundaries on squared-paper tracings; casein areas were delineated by extrapolation of the sides of the peaks, serum areas by drawing ordinates from the minima (*cf.* Figure 3). The areas were then expressed as μg. N per individual component of casein or serum by taking the appropriate percentage of casein nitrogen (total nitrogen minus noncasein nitrogen) and serum protein nitrogen (total serum nitrogen minus nonprotein nitrogen), as determined by Rowland's procedure. Casein peaks were designated α, β, and γ, in the customary manner (6, 13); serum peaks were numbered 1 to 8 as suggested by Larson and Rolleri (15) and the Committee on Nomenclature (6), but Peaks 3, 5, and 8 (proteose peptones) were ignored. Good electrophoretic separation of the serum proteins was not always obtained, but the data are preferable to those obtained by earlier methods of chemical separation (3).

RESULTS

Composition. Results of the analyses of these milks are shown in Figures 1 and 2, and Table 2. Most components were present in higher concentrations in early- and late-lactation than in mid-lactation milk. Exceptions to this general rule were: β-casein, which was normal in early-lactation and tended to be low in late-lactation milk; soluble inorganic phosphorus, which was low in late-lactation milks; and total citrate, soluble citrate, and soluble calcium, which tended to decrease progressively throughout the lactation period.

In general, milks from both cows showed the same trends in composition for each component studied. However, the composition of milk from Cow No. 1 was much more variable than that from Cow No. 2. Also, the increase in concentration towards the end of the lactation period tended to be more

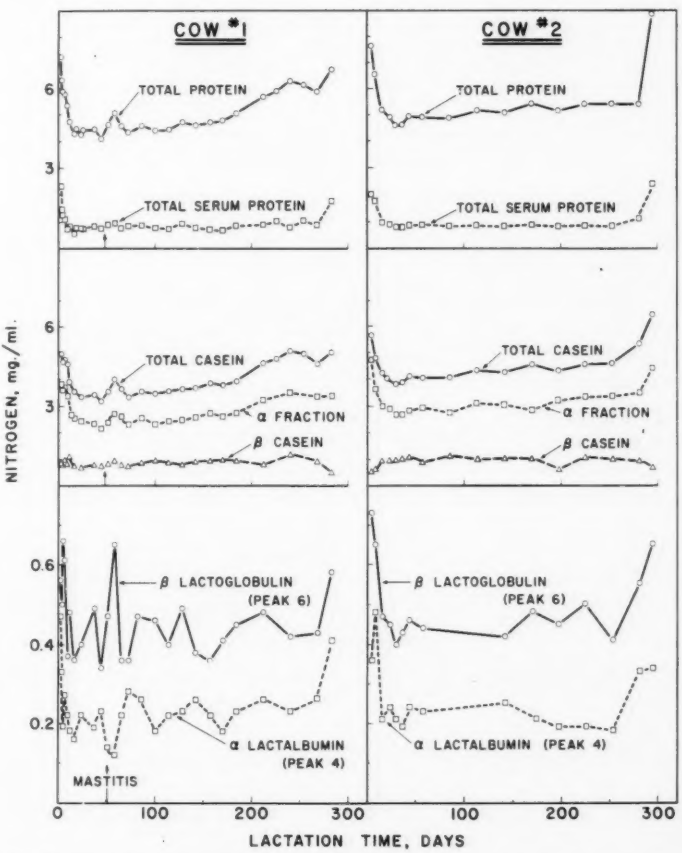

FIG. 1. Changes in concentration of various milk proteins during the lactation cycle.

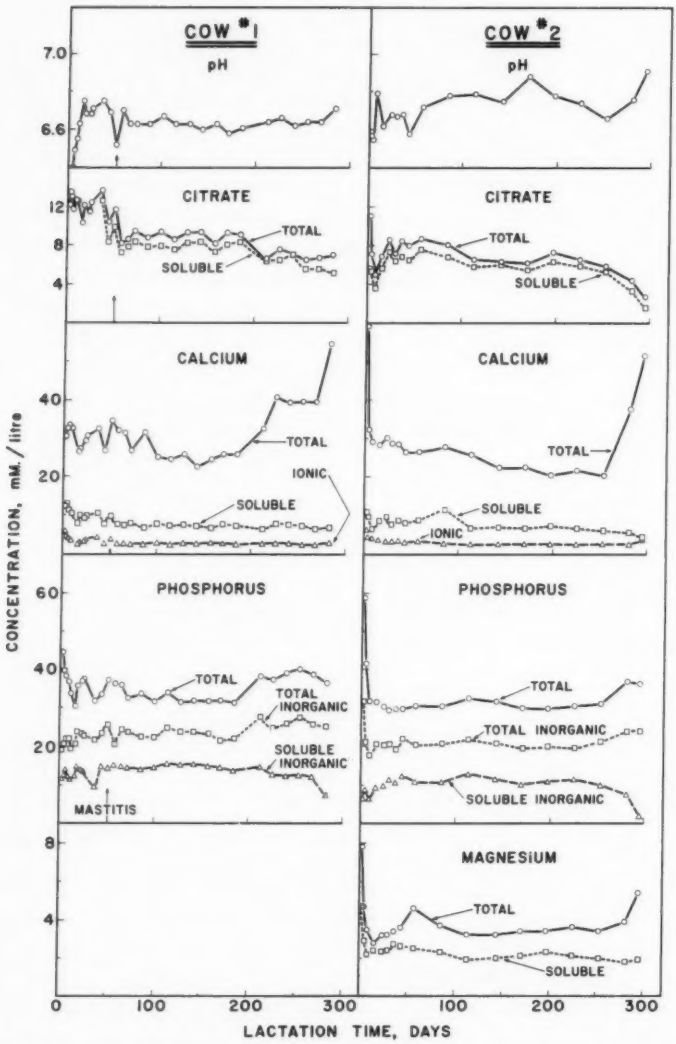

FIG. 2. Changes in concentration of various inorganic constituents during the lactation cycle.

abrupt, for most components, in milk from Cow No. 2 than in that from Cow No. 1.

Correlations between some of the protein components are given to Table 3 [*cf.* (2, 25)].

Two anomalies in the protein composition of very late-lactation milks were noted: the concentration of γ-casein exceeded that of β-casein in the last sample

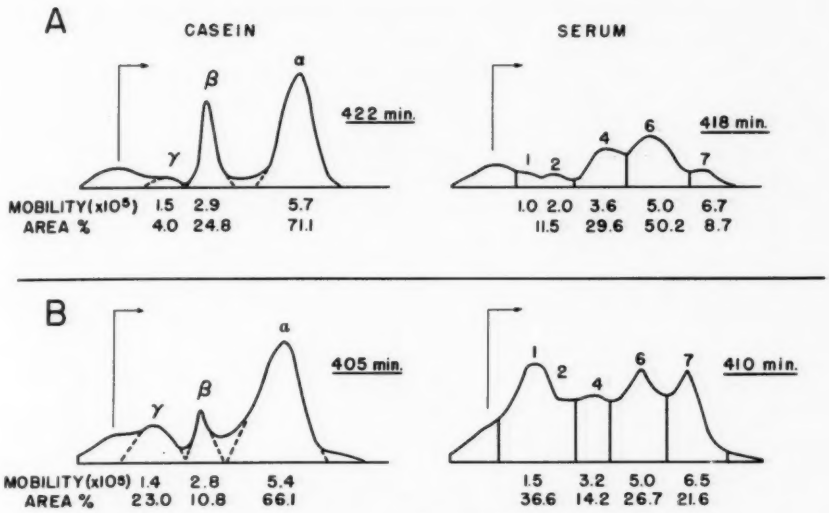

FIG. 3. Electrophoretic pattern for (A) mid-lactation (142nd day) and (B) very late-lactation (295th day) milk from Cow No. 2.

from both cows (Figure 3) and the concentration of blood serum albumin (Peak 7) exceeded that of α-lactalbumin (Peak 4) in the last sample from Cow No. 2 (Figure 3).

Variation of heat stability with pH. Small changes in pH markedly affected the heat stability of raw milk from both cows (Figure 4), although the pH sensitivity of milk from Cow No. 1 was considerably greater than that of milk from Cow No. 2. Both milks usually exhibited a maximum [2] heat stability around pH 6.6 to 6.7 and a minimum around pH 6.7 to 6.9. Further additions of alkali increased the heat stability to very high values. The high pH-sensitivity of milk from Cow No. 1 was observed consistently during three consecutive lactation periods, and similar results were obtained with milk samples from

[2] For convenience, the maximum obtained in this pH range is referred to throughout this paper as the maximum heat stability, although higher values were obtained as the pH was raised above 7.0.

TABLE 3
Correlations between protein components of milk

Components	Correlation coefficients		
	Cow No. 1	Cow No. 2	Combined data
Total casein vs. α-casein	0.97[a]	0.97[a]	0.97[a]
Total casein vs. β-casein	−0.15	0.24	−0.10
Total casein vs. α-lactoglobulin	0.52[a]	0.83[a]	0.65[a]
Total serum protein vs. α-lactalbumin	0.81[a]	0.78[a]	0.79[a]
Total serum protein vs. β-lactoglobulin	0.62[a]	0.91[a]	0.75[a]

[a] Significant to 1% point.

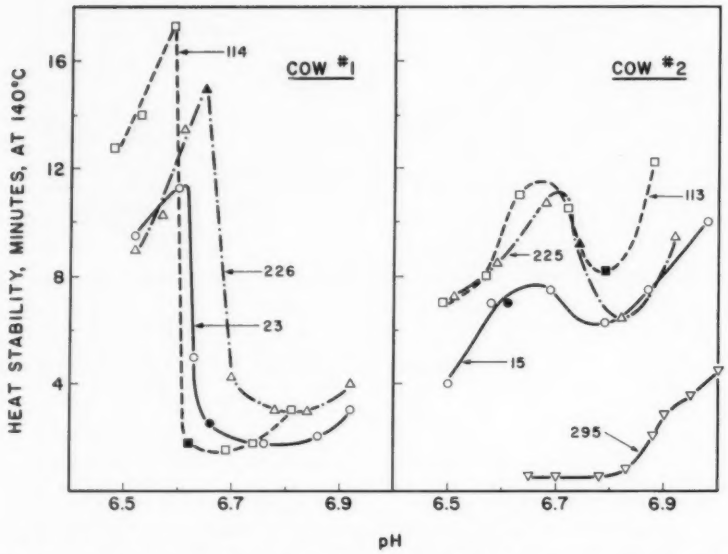

FIG. 4. Effect of pH on the heat stability of milk. Figures beside curves—days of lactation; solid points—original milk; open points—modified milks.

other cows. Samples of bulk milk tended to give results similar to those for milk from Cow No. 2. With Cow No. 1, the shape of the curve was relatively consistent throughout the lactation period, but the pH at which the maximum occurred was not constant. Interpolated maxima were found from pH 6.59 to 6.68 during the mid-lactation period, with an extreme range from 6.52 (fourth day) to 6.72 (283rd day). With Cow No. 2, the pH-sensitivity was absent or insignificant (less than 0.5-min. difference between the maximum and minimum) until the 15th day, varied between 1.5 and 6 min. during the mid-lactation period, and decreased to insignificance again by the 295th day. The pH at which the maximum occurred varied from 6.65 to 6.79 during the mid-lactation period, with a high of 6.93 on the 281st day.

The heat stability of the untreated milk from both cows varied widely during the lactation cycle, but the maximum heat stability was less variable, particularly with Cow No. 2 (Figure 5A). The amount of acid or base required to adjust the milk to the pH of maximum heat stability also varied from sample to sample (Figure 5B). The buffer capacity of all samples was essentially linear throughout the narrow pH range studied, but varied between 0.03 and 0.06 pH unit per millimole of added acid or base.

In agreement with published results (32), the heat stability of the samples was also affected by small additions of calcium chloride or of phosphate. However, additions of calcium chloride increased the acidity of the samples by about 0.04 pH unit per m*M* Ca/liter, and with most samples this pH change was sufficient to explain the change in heat stability. Data for a few samples suggested

that calcium had a specific effect in addition to its effect on pH, but the evidence was not conclusive. Addition of phosphate to samples which would attain maximum heat stability by an increase in pH (i.e., requiring added hydroxide) consistently increased the heat stability, and this change was not dependent upon pH. However, the increase in heat stability achieved by addition of phosphate never exceeded 2 min. Addition of phosphate to samples which would attain maximum heat stability by a decrease in pH (i.e., requiring added acid) usually decreased the heat stability, and this change was also independent of pH.

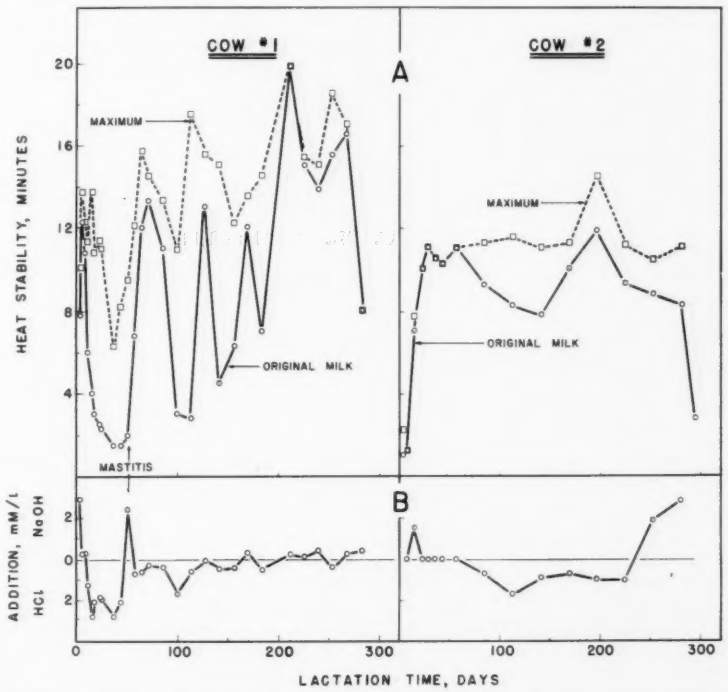

FIG. 5. A. Changes in heat stability and in the maximum heat stability (obtained by pH adjustment) during the lactation cycle. B. Amount of acid or base required to adjust the milk to the pH of maximum heat stability.

DISCUSSION AND CONCLUSIONS

In general, the data on composition of these two milks are in agreement with those previously published (8, 12, 21, 33), although the decrease in citrate content with increasing time of lactation may be unusual. Low β-casein and high albumin values have been reported for regression milks (20), but these are not comparable to the present late-lactation milks collected at least 60 days before recalving. To the best of our knowledge, the high γ-casein content of late-lactation milk has not been reported previously.

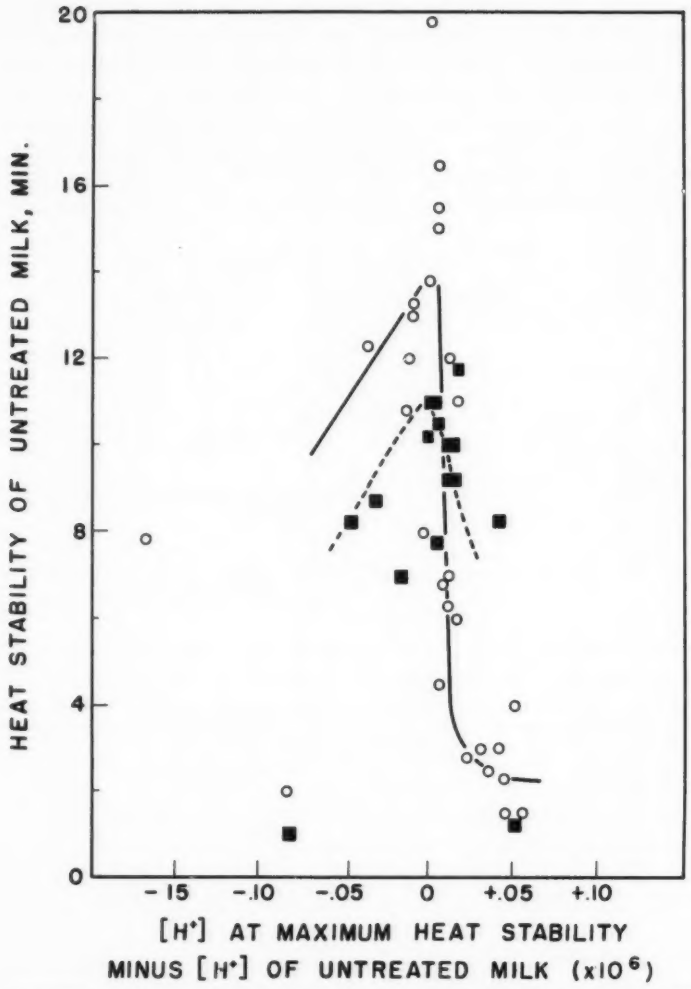

FIG. 6. Relation between heat stability of the untreated milk and the adjustment in [H^+] required to give maximum heat stability. Open circles—Cow No. 1; filled squares—Cow No. 2.

The heat stability of these milks was highly dependent on the pH of the individual milk; with some samples from Cow No. 1 a pH change of 0.02 unit could increase or decrease heat stability by as much as 10 min. (Figure 4). Somewhat similar results were obtained by Benton and Albery (5) in a study of the effect of buffers, and by Webb and Holm (32). Sommer and Hart (28) and Fay [quoted by Sommer and Hart (29)] also observed that with some samples, heat stability increased and then decreased on addition of acid. How-

ever, none of these workers added both acid and base to one sample of milk. The present results suggest that the two types of milk previously distinguished (31, 32) may differ only in the relation between the pH of the original milk and that at which a maximum heat stability occurs.

In agreement with published results (7, 10, 23, 34), consideration of the combined data for both cows showed that the inorganic components of the milk were not significantly correlated with the heat stability of the untreated milk. Furthermore, none of the whey protein fractions for which data were not previously available was significantly correlated with the heat stability.

The heat stability of the untreated milk appeared to be determined by the difference between the hydrogen ion concentration of the untreated milk and that to which the sample had to be adjusted to obtain maximum heat stability, i.e., untreated milks with low heat stabilities required more acid (or alkali) to attain maximum heat stability than did those with high stabilities. The relation between the $[H^+]$ difference and the heat stability of the untreated milk (Figure 6) showed the same complexity as the relation between pH and heat stability of a mid-lactation sample (Figure 4). Of the nine samples from Cow No. 1 with heat stabilities of 4 min. or less (Figure 5), eight required an increase in $[H^+]$ of at least 0.023×10^{-6} *M* (a pH shift of 0.04 or more in the range involved) to give maximum heat stability, and one required a decrease in $[H^+]$ of 0.083×10^{-6} *M*. The ten samples giving maximum heat stability with a change in $[H^+]$ of less than 0.01×10^{-6} *M* had an average original heat stability of 12.6 min.

The possible relations between the maximum heat stability obtained by pH adjustment and the composition of the milk were examined in considerable detail, and the significant correlations plus a few others of interest are recorded in Table 4. With Cow No. 1, the original heat stability was not significantly correlated with any component tested, but the maximum heat stability was

TABLE 4

Correlations between heat stability and the calcium/phosphate ratios

Components	Correlation coefficients		
	Cow No. 1	Cow No. 2	Combined data
Heat stability of original milk versus			
(1) Total soluble Ca/soluble inorg. P	−0.24	−0.21	−0.23
(2) Ionic Ca/soluble inorg. P	−0.20	−0.48[a]	−0.26
(3) $\frac{\text{Soluble Ca + soluble Mg}}{\text{Sol. inorg. P + sol. citrate}}$	.....	−0.63[b]	.....
(4) As for (3)[c]	.....	−0.72[b]	.....
Maximum heat stability versus			
(1) Total sol. Ca/sol. inorg. P	−0.62[b]	−0.14	−0.44[b]
(2) Ionic Ca/sol. inorg. P	−0.63[b]	−0.36	−0.41[b]
(3) $\frac{\text{Soluble Ca + soluble Mg}}{\text{Sol. inorg. P + sol. citrate}}$	.....	−0.54[a]	.....
(4) As for (3)[c]	.....	−0.81[b]	.....

[a] Significant to 5% point.
[b] Significant to 1% point.
[c] Omitting sample from 295th day.

significantly correlated with the ratio of Soluble Ca/Soluble Inorganic P, and of Ionic Calcium/Soluble Inorganic P. With Cow No. 2, the heat stability of the untreated milk was correlated (5% point only) with the Ca^{++}/Soluble Inorganic P ratio, but the correlation between maximum heat stability and this ratio was slightly below the 5% significance level. When the data for both cows were considered together, the maximum heat stability correlated significantly (1% point) with the ratio of Soluble Calcium or of Ionic Calcium/Soluble Inorganic Phosphorus. With Cow No. 2, sufficient data were obtained to permit calculation of the Soluble Calcium + Soluble Magnesium/Soluble Inorganic Phosphorus + Soluble Citrate ratio, and it was found that this ratio was significantly correlated with both the original and the maximum heat stability (Table 4). All correlations were negative, i.e., increasing calcium (or calcium plus magnesium) relative to phosphate (or phosphate plus citrate) decreased heat stability.

Although based on too few samples to be conclusive, these results indicate that further study of the relation between the composition of the milk and the maximum heat stability obtainable by pH adjustment is desirable. They also indicate that extremely accurate pH determination and control is necessary in tests of the effects of added salts, forewarming, etc., on the heat stability of milk.

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HIGH TEMPERATURE–SHORT TIME STERILIZED EVAPORATED MILK. II. LABORATORY TECHNIQUES FOR THE PREPARATION AND STUDY OF STERILE EVAPORATED MILK

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SUMMARY

Laboratory techniques for the preparation and study of high temperature–short time sterilized evaporated milk are described. A bomb, falling-ball microviscometer combination, easily constructed in large numbers, is employed for containing samples during sterilization and storage, and for following changes in viscosity. Normally, the instrument constant for the microviscometer is determined by standardization against liquids of known viscosity. This procedure is too time-consuming and tedious for research on evaporated milk. For such research, studies show that the instrument constant may be calculated expeditiously, with sufficient accuracy, with the help of the following equations:

$$C_M = f\left(\frac{d}{D}\right) d^2 \cos\theta; \text{ and}$$

$$f\left(\frac{d}{D}\right) = 0.0178\text{-}0.0217\,\frac{d}{D}.$$

C_M is the instrument constant, d and D are, respectively, the diameter of the ball and tube, $f\left(\frac{d}{D}\right)$ is a function of the ratio between the diameters, and θ is the angle of inclination of the tube. Forewarming and evaporation are carried out in a rotating film evaporator, and sterilization in a thermostatically controlled oil-bath. An electrically operated metronome is employed for controlling the time of sterilization. Small-scale homogenization is facilitated by the employment of a diesel nozzle tester and nozzle combination modified to function as a hand-homogenizer. The application of the techniques to the study of gelation is illustrated in studies on milks containing added calcium salts and phosphates. Wide variations in $[Ca^{++}]$ and $[HPO_4^{=}]$ influenced storage stability to only a small degree, with a small advantage accruing to the use of limited quantities of calcium salts. False body, but not the minimum viscosity attained during storage, increased markedly with increasing $[Ca^{++}]$. Repeatability of results was quite satisfactory. In one series of experiments based on the same sample of whole milk, a mean storage life of 84 days was observed; the limits for the mean at a 95% confidence level were $\pm$ 5 days.

In the production of high temperature–short time (HTST) sterilized evaporated milk, sterilizing temperatures of approximately 137° C. are employed for periods of time ranging from seconds to tens of seconds. Both the color and flavor attributes of freshly prepared HTST sterilized evaporated milk compare favorably with the corresponding attributes of market milk. Because it is capable of storage without refrigeration, HTST evaporated milk would be a worthy competitor of market milk, were it not for the fact that it tends to set to a renneted milk-like structure on short storage.

Sterile milk for gelation studies is usually manufactured on a pilot plant or plant scale. Cans of milk are opened periodically and viscosity measurements

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are made according to accepted viscometric practice. Such studies, although they have the merit that results are immediately translatable into plant practices, have a number of disadvantages, particularly from the point of view of the chemist who would prefer to work in his laboratory dissociated from plant and pilot plant operations. In order to bring research studies on HTST sterilized milk entirely within the purview of the laboratory chemist, techniques have been developed which permit the carrying out on a small laboratory scale of many, if not all, of the processing steps: forewarming, homogenization, concentration, and sterilization. These techniques will be described in this paper and their application and advantages will be discussed. They are based on the employment of the bomb microviscometer described in the first paper of this series (4).

APPARATUS AND METHODS

Selection of tubes and beads. Certain modifications were made in the construction and operation of the bomb viscometer. For studies on evaporated milk, tubes approximately 3.5 cm. long, 0.445-0.455 cm. in bore, and 0.020% maximum bore gradient were selected on the basis of microscopic examination. Corresponding to each tube a bead was selected the diameter of which fell in the range of 0.62-0.75 times the diameter of the tube. The size range of the beads was narrowed by the use of specially constructed sieves consisting essentially of perforated brass plates about 1 in. diameter and $\frac{1}{16}$ in. thick. Numerous perforations in one plate were made with a 0.012-in. drill. Alternately, a simplified selector was used comprising the following: an open-end box constructed out of $\frac{1}{4}$-in. plastic each of the sides of which had four vertical grooves; a glass plate resting on the plastic base and hugging the sides of the box; two razor blades sliding in and out of two sets of appropriate grooves, and forming the ends of the box; a third blade the center portion of which was raised 0.012 above the glass base plate by means of strips of gauge plate, and a fourth blade the center portion of which was raised 0.011 in. The raised blades constituted partitions between which beads 0.275-0.305-mm. diameter were trapped. These beads were noticeably free from defects, inasmuch as they were representative of beads which rolled freely at a small angle of inclination. The partitions were pressed firmly against the strips of gauge plate by means of rubber bands. Clean and dry surfaces were essential for the proper functioning of the selector.

Forewarming and concentration. Forewarming of small milk samples at the temperature of boiling water for 15 min. was effected in a laboratory rotating film evaporator in the presence of air or in a current of nitrogen, as the occasion demanded. The forewarmed sample in the evaporator was concentrated in vacuo to 26% solids. Concentration was usually carried out to a point slightly beyond the desired concentration and a final volume adjustment was made with water. From knowledge of the initial solids concentration and the weight of the sample before and after concentration, the final solids concentration was controlled. Additional agitation was imparted to the contents of the flask by means of a glass baffle moving freely within the flask. Following its concentration and standardization, the sample was passed through a fine mesh wire filter screen.

Sterilization. Small samples of concentrated milk were deaerated momentarily in vacuo. Bomb viscometers were loaded with deaerated samples and mounted in fine wire stirrups. The mounted samples were plunged into an oil-bath at 137.4 ± 0.15°, and after being held for 15 sec. they were removed and plunged into water at room temperature. Timing was made by the use of an electrically operated metronome. The bringing-up time is not known with certainty; an intelligent guess based on heat conductivity theory would require about 0.5 sec. to bring the temperature at the center of the viscometer to within 0.2° of the temperature of the bath.

The oil-bath consisted of a deep fryer (located within a thermostatically controlled air bath) equipped with a stirrer and an auxiliary heater with controlled heat input operating through an electronic relay. Control of the heat input to both the oil, and the surrounding bath, and immersion of the samples on all occasions at the same point in the heating and cooling cycle allowed for excellent reproducibility with respect to the important variable-heat treatment.

Forewarming and concentrating in microviscometers. Samples may be forewarmed and concentrated in microviscometers. Forewarming may be conducted either in the presence of air or in vacuo. Although the procedure for concentration is not complicated, its use is recommended only under special conditions, for painstaking effort is required, samples are lost occasionally and, finally, the concentrate, because of the employment of quiescent evaporation, is not comparable with the concentrate obtained in turbulent evaporation. Special conditions which justified forewarming and concentrating in microviscometers were met, for example, in the study of additives when these were available only in very small quantities or when they were heat-labile. Appropriate formulae for calculation of the degree of concentration in terms of thread length were easily derived.

Measurement of viscosity. Viscosity measurements were made according to the method described in the preceding paper (4). It was found expedient to take as a starting point in the motion of the bead a point reasonably close to the bottom of the viscometer. Consequently, the zero point was located at approximately 0.4 cm. above the base of the thread. The time required by the bead to move first 0.75 cm., and then 0.375 cm., was noted. Two stop watches were employed for the purpose. At times the flow characteristics of milk were not adequately defined, even for practical purposes, at a single applied shearing stress, and to meet such contingencies a plastometer was constructed by means of which the applied force could be varied in steps from approximately one to 35 times gravity. This plastometer consisted of a quick-acting constant-speed motor equipped with reduction gears and pulleys driving a gear-actuated turntable. The turntable, a plastic disk 30 cm. in diameter, contained 16 radial groove-like lodgements for the viscometers. Inasmuch as the velocity of the bead moving through a truly viscous fluid is proportional at any moment to the applied centrifugal force, the viscosity of the fluid could be calculated if, in addition to the parameters associated with the viscometer, the parameters associated with the centrifuge were known. The plastometer also lent itself to the

characterization of the thixotropic behavior exhibited by concentrated milks at one stage or another of their storage life.

Storage of samples. Samples were stored in a constant temperature room maintained at 30° C.

Microviscometers were accommodated in corks with drilled spaces. The corks fitted into plastic tubes which served to shield the viscometers from accidental breakage. During storage it was found desirable to rotate the microviscometer at about 5 r.p.m., with its axis always in a horizontal position, to prevent the complicating effects of creaming and sedimentation in the study of gelation.

RESULTS AND DISCUSSION

Determination of the instrument constant. The instrument constant, if maximum precision is desired, is determined by measuring the velocity of the bead in a liquid of known viscosity and density (3). This procedure in studies on evaporated milk is too time-consuming and tedious, in view of the large number of viscometers which are employed. Fortunately, precision can be sacrificed in the interest of expediency in such studies. Because of the non-Newtonian character of sterile concentrated milk and because, in storage studies, knowledge of changes in viscosity supersede in importance accurate knowledge of the initial viscosity, that is, the viscosity of the unsterilized sample, conditions of measurements of the initial viscosity can be relaxed.

The interpretation of viscosity data on evaporated milk should be made in the light of the anomalies known to belong to these products (1, 5). The dependence of the viscosity coefficient on shearing rate, on the history of the sample, and on mechanical disturbance are well known. A single measurement made under carefully defined conditions is useful at best as an index defining an order of magnitude which can be compared with other indices obtained in the same manner and related to what may be described as the sensible body or consistency of the product.

The instrument constant may be calculated with sufficient accuracy if the two easily measured parameters, the diameters of the bead and the tube, are shown. The function $f\left(\frac{d}{D}\right)$ of the ratio between the bead and the tube diameter is calculated for various values of the ratio $\frac{d}{D}$ by means of the following equation (6):

$$f\left(\frac{d}{D}\right) = \frac{\eta_m v}{d^2 (\rho_b - \rho_m) g \cos \theta} \qquad (1)$$

η_m and ρ_m are the viscosity and density, respectively, of unsterilized evaporated milk, v is the velocity of the bead, ρ_b its density, d its diameter, g is the gravitational constant, and θ is the angle of inclination of the viscometer. The parameters on the right-hand side are easily measured. Over a limited range of $\frac{d}{D}$ values, $f\left(\frac{d}{D}\right)$ may be plotted as a linear function of $\frac{d}{D}$. This has been done in Figure 1.

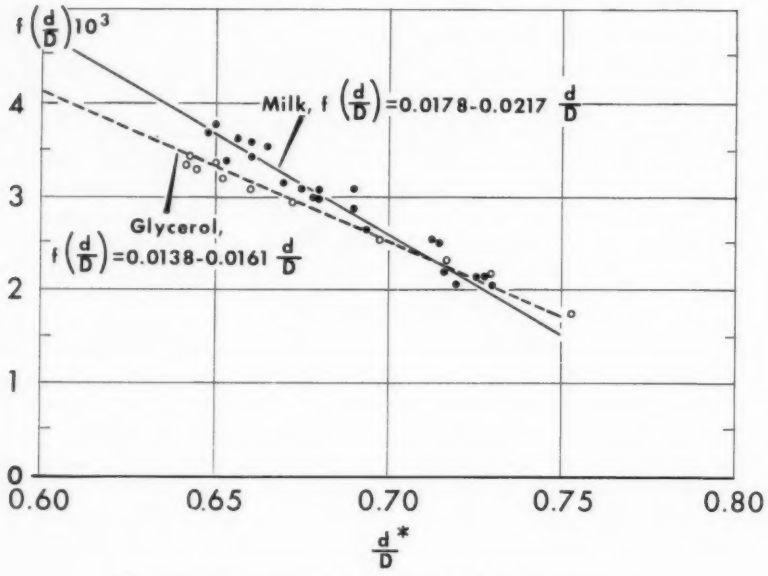

FIG. 1. The relationship over a limited range of values between the function $f\left(\frac{d}{D}\right)$ of the ratio between the bead and tube diameters and the ratios $\frac{d}{D}$ The curve referring to concentrated unsterile milk (26% solids) does not coincide with the one referring to glycerol solutions.

Two lines, the constants of which were calculated by the method of least squares, are shown; one refers to measurements on glycerol solution, the other to measurements on concentrated milk containing 26% solids. The fact that the lines do not coincide means that milk is behaving anomalously, a consequence possibly of its non-Newtonian character. To calculate the instrument constant, C_m, the value of $f\left(\frac{d}{D}\right)$ for milk corresponding to the ratio $\frac{d}{D}$ is read from the graph and inserted in Equation 2, thus:

$$C_m = f\left(\frac{d}{D}\right) d^2 g \cos\theta \qquad (2)$$

The viscosity of a milk sample contained in the viscometer is then given by Equation 3, thus:

$$\eta_m = \frac{C_m(\rho_b - \rho_m)}{v} \quad (\text{v} = \text{bead velocity}) \qquad (3)$$

Equations 2 and 3, when combined with the respective values 981 dynes, 0.983, 2.42, and 1.05 for g, cos θ, ρ_b and ρ_m substituted therein yield Equation 4, thus:

$$\eta_m = 1{,}340\, f\left(\frac{d}{D}\right) \frac{d^2}{v} \text{ (for evaporated milk with } \rho_m = 1.05) \qquad (4)$$

TABLE 1
Calculation of viscometer constant and viscosity

Tube diameter (cm.)	Ratio between [a] diameters	$f\left(\frac{d}{D}\right) \times 10^3$	$\frac{C \times 10^3}{cm^3/t^2}$	Viscosity calculated (centipoises)
0.0427	0.720	2.15	1.97	3.94
0.0427	0.715	2.28	2.06	3.50
0.0427	0.670	3.27	2.60	3.88
0.0427	0.716	2.25	2.05	3.91
Mean				3.81
Standard deviation				0.21
Viscosity (transpiration type viscometer)				3.78

[a] Ratio between bead and tube diameters.

Table 1 shows the results of an experiment in which milk of known viscosity was loaded into four viscometers varying in their instrument constants. The values of $f\left(\frac{d}{D}\right)$ were obtained from Figure 1, C was calculated by means of Equation 2, and from knowledge of these values, and the values of v and d, the viscosity of the product was calculated by means of either Equation 3 or 4. The viscosity of the milk as measured with a transpiration type viscometer operating at a maximum shearing stress of 160 dynes per cm.^{2} was 3.78 centipoises. The viscosity as measured with the microviscometer averaged 3.81 with a standard deviation of $\pm$ 0.21 centipoise. This constitutes good agreement.

Viscosity changes attending sterilization and storage. Concentrated milks sterilized in microviscometers are apt to exhibit the phenomenon of false body to a greater extent that the same milk sterilized under the conditions of turbulent flow. The following sequence of changes is usually, although not always, observed in studies with the bomb microviscometer; the viscosity first decreases (a characteristic of false body) more or less over what in some cases is a considerable period of time; it then remains measurably constant for a storage interval the duration of which is not clearly governed by the rate of change which precedes and follows; the apparent viscosity begins to rise slowly at first, then more rapidly until it becomes nonuniformly infinite throughout the sample.

False body may manifest itself as a soft gel-like structure immediately after sterilization, and under some conditions this structure, although not necessarily undesirable, may persist for long periods of time. Again, a highly thixotropic structure, one yielding to the application of mild shearing stresses, may develop immediately after sterilization and persist through the major portion of the shelf life of the product. It is understandable in view of the foregoing that the point at which the apparent viscosity becomes infinite can not be taken seriously as an end point indicating the end of the storage life of the sample.

A much more satisfactory end point becomes available if the criterion for judging deterioration is not gel formation but rather instability in body represented by rapid rate of increase in apparent viscosity.

Viscosity measurements immediately following the sterilization step are made on unstirred and stirred-out samples. Samples may exhibit at times

structure and complete or partially reversible coagulation following sterilization. Stirring-out of such samples is continued until segregation along the milk column is eliminated. A number of techniques for stirring-out are available. The position of the liquid thread may be reversed centrifugally a number of times. More drastic but more difficult to control is the technique of applying momentarily a microflame to the beaded base of the microviscometer. Localized heating violently disrupts the thread into fragments which are reunited by centrifuging. Finally, the technique of alternately freezing and thawing of the contents of the tube in an acetone-CO_2 bath followed by centrifuging to reverse the position of the liquid thread a number of times serves to stir out structure drastically.

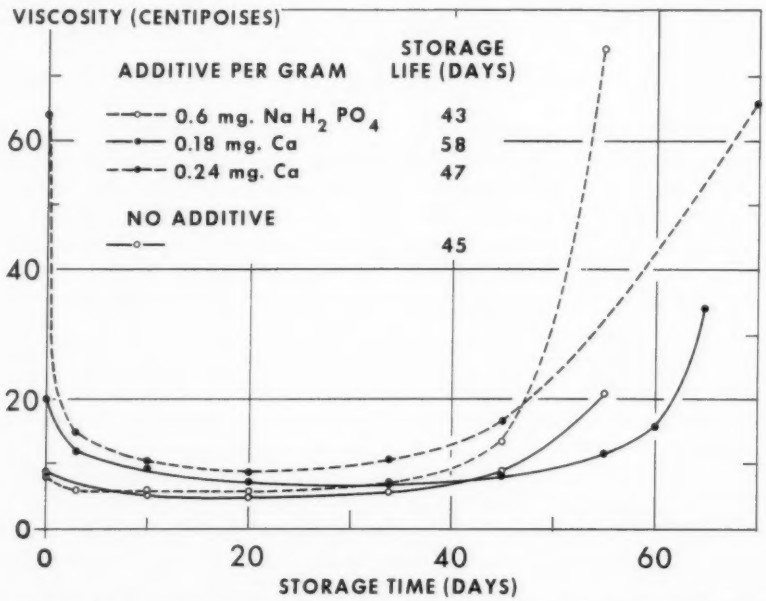

FIG. 2. Typical curves illustrating storage effects on viscosity curves obtained in studies on high temperature–short time sterilized milks with laboratory techniques employing quiescent heating and cooling. Curves also show effects of added calcium and phosphate on storage life.

Figure 2 shows a number of typical curves relating apparent viscosity and storage time. The milk employed in this study was a foam-dried product reconstituted to contain 3.5% fat and 13% total solids. It was then concentrated to 30% total solids. To each of two 1-g. samples of concentrate, 0.3 ml. of the following were added: water, 0.03 *M* $CaCl_2$, 0.04 *M* $CaCl_2$, and 0.4% Na_2HPO_4. The various samples were introduced into bomb microviscometers and sterilized for 15 sec. at 137.4° C. The viscosity of the samples prior to sterilization was 3.9 centipoises.

Although the curves in Figure 2 are intended to convey primarily some idea of the type and character of changes observed during storage (and these resemble the curves obtained in studies in which conventional techniques are employed), there are the following additional points of interest:

(1) The viscosities of the samples immediately after sterilization were related to heat stability but not to storage stability.

(2) Graininess, such as the sample containing added 0.04 *M* $CaCl_2$ showed, disappeared during storage. Such samples, therefore, could hardly be classified as defective.

(3) Both the minimum viscosity acquired by the samples, and the storage stability, were influenced only to a small degree by wide variaiton in (Ca^{++}) and $(HPO^{=}_4)$.

(4) The sample containing added phosphate showed very little false body, reached a minimum viscosity value quickly, and set once the minimum viscosity was exceeded, to a rather firm structure exhibiting macroscopically visible curd particles.

(5) The addition of $CaCl_2$ is conducive to the development of false body in proportion to the quantity added. The degree of false body reached extremely large proportions much larger than Deysher *et al.* (2) have reported for long-hold evaporated milk.

(6) The structure which developed during the storage of the high calcium concentrate was finer and possessed a higher degree of thixotropy than the structure which developed in the control, and high phosphate concentrate.

(7) The concentrates began to increase in viscosity at approximately 20 days, irrespective of whether calcium or phosphate had been added; whatever small differences in storage life were observed were related to the rate at which body developed during the period of viscosity increase.

Reproducibility of results. To obtain reproducible results, processing conditions must be adequately controlled, and viscometry conditions must be standardized. Considering the large number of variables involved, the long duration of an experiment, and the complex nature of the coagulation phenomena, one may view with satisfaction the results obtained with laboratory techniques.

The data in Table 2 are illustrative. They show the results of an experiment in which samples of concentrated milk containing 7.3% fat and 26% total solids were sterilized at 137.4° C. for 15 sec. All samples were derived from the same batch of forewarmed milk. Three concentrates, each prepared to contain the same concentration of solids, were sampled, two in duplicate and one in triplicate. Viscosities were measured before and after sterilization and during storage.

Variations between concentrates were observed corresponding to a standard deviation in presterilization viscosity of 0.1 centipoise. The possibility that variations in storage life were related to the slight variations in presterilization viscosity between samples could not be ruled out on the basis of variance analysis.

TABLE 2
Data pertaining to the storage life of samples prepared from the same batch of whole milk

Sample [a]	Viscosity before sterilization	Viscosity after	Viscosity minimum	Time to reach minimum	Storage life
	(centipoises)	*(cp.)*	*(cp.)*	*(days)*	*(days)*
A1	3.5	15.4	4.9	60	95
		13.7	4.8	58	92
A2	3.7	17.1	5.2	64	92
		17.5	4.6	64	90
A3	3.6	15.2	4.7	60	94
		17.5	5.0	64	98
		14.8	4.3	64	104
Mean	3.6	15.9	4.8	62	94
Limits for mean (95% confidence)	±0.1	±1.7	±0.3	±3	±5
Standard deviation	0.1	1.5	0.3	3	5
Correlation coefficient		0.6	0.06	0.6	0.17

[a] Samples represent three concentrates from same milk.

Relatively poor correlation as measured by the square of the correlation coefficient was observed between concentrate sample, and both storage life and minimum viscosity variations. A more marked correlation was observed between concentrate sample, and viscosity variations in freshly sterilized milk. The variations noted were not significant from a practical point of view. The mean storage life was 84 days; the limits for the mean at a 95% confidence level were ± 5 days.

Thermal history and concentration are two important variables which require careful control in order to obtain reproducible results. Milk as a biological fluid varies in composition from day to day and from place to place and, consequently, the effects of external variables are best evaluated statistically if time permits. In this respect, the laboratory techniques which have been described can contribute much to a broadening of the base of evaporated milk research, permitting thereby the accumulation of the large bodies of data required for adequate statistical evaluation.

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RINDLESS, SWISS-TYPE CHEESE. I. METHOD OF MANUFACTURE IN BLOCK FORM[1]

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SUMMARY

Rindless Swiss-type cheese was made in 20-lb. blocks by modifying the procedure previously used in making the cheese as 5-lb. wheels. Manufacture of the cheese in 20-lb. blocks did influence eye formation. Less *Propioni-bacterium shermanii* culture was used and the eyes formed were larger and more characteristic of Swiss cheese as compared to those found in the 5-lb. wheel.

The rate of acid development during manufacture was important, since those blocks with a pH of 5.05 to 5.10 after pressing overnight had the most desirable eye formation. At a pH above 5.20, the cheese had a tendency to develop too many eyes. To obtain the proper rate of acid development during manufacture, it was necessary to use a longer ripening period and a longer pressing of the curd mass before cutting into blocks to be wrapped and pressed overnight.

The cheese was cured in an unsealed, plastic film wrapper held in place with plastic tape. The cheese had a mild flavor at 3 to 4 mo. of age, whereas that held for longer periods, up to a year, had a more definite Swiss flavor.

The method of manufacture of Iowa Swiss-type cheese was first published some 20 years ago (8). Since that time various modifications in method of manufacture have been reported (3, 10, 11, 12). Originally, the cheese was made and cured as a wheel that weighed 23-25 lb. (8). Later, the size was reduced; first to 15-16 lb. (3), and then, about 1939-1940, to 5 lb. (9). The cheese, after salting, was held at 55-65° F. to dry and to allow eye formation to take place before waxing.

In recent years, emphasis has been placed on the manufacture of cheese in the form of a rindless block that can be cut into small portions, packaged and marketed with a minimum of waste. The state dairy experiment station at Hillerod, Denmark, has published a report of their work on the manufacture of Samso and Maribo cheese as rindless blocks (1). The present investigation was undertaken to study methods of manufacture of Iowa Swiss-type cheese in the form of a rindless block that would lend itself to cutting and packaging in consumer-sized portions.

EXPERIMENTAL PROCEDURE

Methods of manufacture previously reported (3,8) were used as a basis for determining the proper method of manufacture of Iowa Swiss-type cheese in rindless 20-lb. blocks.

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Fat tests on milk and whey were carried out by the Babcock method (7). The fat tests on the cheese were carried out at first by the official method for cheese (2), but it was found that closer agreement between duplicate samples could be obtained if the method was modified by weighing the cheese into the fat-extraction flask as in the official test for fat in dried egg (2), and then digesting with the mixture of water and NH_4OH, thus eliminating the necessity of transferring the contents of a beaker to the fat-extraction flask. Chlorides were determined by the official method (2), while the moisture test was carried out by drying a 4- to 5-g. sample of cheese under vacuum at 105-110° C. for 5.5 hr. in an aluminum dish containing dry sand.

In the first lots, the pH of the cheese was determined by a Leeds and Northrup quinhydrone electrode. With the commercial lots, a Beckman Zeromatic pH meter with a glass electrode and a sleeve-type calomel reference electrode was used.

All evaluations of body, texture, flavor, and eye formation were made by at least three judges.

RESULTS AND DISCUSSION

The various modifications carried out in establishing the most desirable manufacturing procedure are listed in Table 1. Grade A mixed herd milk from the University creamery testing 3.7-3.8% milk fat was used. The milk was pasteurized at 162° F. for 16 sec., cooled and standardized, in the cheese vat, with pasteurized fluid Grade A skimmilk to a fat content ranging from 2.5 to 2.9%. Milk standardized to 2.5-2.6% fat and a C/F ratio of 0.93 ± 0.01 produced cheese with the desired fat content of 44% on a dry basis. Iowa Swiss-type cheese made from milk standardized to 2.7 to 2.9% milk fat had a slightly softer body because of the higher fat content. No other characteristics attributed to the use of milk with the higher fat content were observed.

The setting temperature of most vats of milk was 88° F. Small variations of 1 or 2° F. above or below 88° F. had little effect, although when the temperature was reduced to 85° F. the coagulation time was prolonged slightly.

TABLE 1

Modifications used in manufacture of block-style, rindless, Swiss-type cheese

Factor studied	Extent of modification
Fat test following standardization *(% milk fat)*	2.5, 2.6, 2.7, 2.8, 2.9
Temperature of ripening and setting milk *(° F.)*	85, 86, 87, 88, 89
Lactic culture added *(%)*	1.20, 1.25, 1.30, 1.40
Titratable acidity development during ripening *(as % lactic acid)*	0.000, 0.010, 0.015, 0.020, 0.025, 0.030
Propionibacterium shermanii added *(ml/1,000 lb. milk)*	15, 25, 40, 60, 80
Temperature of hot water added to reach cooking temperature *(° F.)*	140, 145, 150, 155
Temperature of cooking *(° F.)*	98, 100, 101, 102
Time curd pressed behind dam after cooking *(min.)*	10-120
Titratable acidity of whey when dam removed and blocks cut *(as % lactic acid)*	0.12-0.65
pH of whey when dam removed and blocks cut	5.05-6.20
pH of block after pressing overnight	4.95-5.30
Length of salting period *(days)*	1-3
Holding period before placing in warm room *(days)*	0-14

The amount of lactic starter culture used, and the ripening time required to bring about a desirable rate of acid production, varied somewhat with the particular starter culture used. In early trials, ripening conditions were adjusted to bring about a titratable acidity increase as high as 0.03%; however, later trials showed that an increase of 0.015% in titratable acidity was sufficient. Addition of 1.25% active starter culture and ripening for 45-60 min. generally was optimum. The use of 1.30 and 1.40% lactic starter was unsatisfactory because of the difficulty in obtaining the proper rate of acid development in subsequent procedures in the manufacturing process. In previous reports of making Swiss-type cheese (3, 8) 1% lactic starter and a ripening period of 20-45 min. was used. Peters (11) used 1% starter with 60 min. ripening but did not report the amount of acid development.

The *Propionibacterium shermanii* was grown in yeast extract, peptone, dextrose broth (0.5% each) at room temperature (70-75° F.) for 24 hr., or until good growth was obtained, and then held at 60-70° F. until used. Typical eye formation occurred with cultures from 1 to 6 wk. of age. Excellent eye formation resulted from the use of 25 to 40 ml. of the propionic culture per 1,000 lb. of milk. At present, 25 ml/1,000 lb. of milk is used. This is about 50% of the inoculum Babel and Hammer reported (3) and one-fourth that found best by Peters and Moore (12).

Cooking at 102° F. was preferred, since cooking at lower temperatures meant a longer cooking time. The water added to reach the cooking temperature usually was at 150° F. If the acid development was somewhat more rapid than desired, it could be retarded slightly by the extra dilution brought about by adding water at 140 to 145° F. to reach the cooking temperature.

One of the major departures from methods previously used in making Iowa Swiss-type cheese was the procedure used in pressing the curd mass behind the curd dam. When the cheese was made in 5-lb. wheels approximately 30 min. was spent, after the curd dam was in place, to drain the whey and fuse the curd into a mass to be cut into individual blocks. Little attention was given to the whey acidity (3, 8, 10, 11). When the cheese was made in 20-lb. blocks, however, the curd normally was held, under pressure, for 60 min. after the whey had drained, before cutting into blocks. With slow acid development the pressing period was increased 15 to 30 min., whereas a shorter period was used when acid development was more rapid than normal. Pressing for 60 min. in the curd dam resulted in a more firmly knit curd mass, important since the 20-lb. blocks of fresh curd were subjected to more stress in handling than 5-lb. wheels.

The desired titratable acidity of the whey from the curd mass when cut into blocks was 0.20-0.25%. The rate of acid development was important, since excessive acid development was associated with a flavor described as slightly cheddary, whereas slow acid production resulted in high moisture and an overset cheese with too many eyes.

The individual blocks of curd were wrapped loosely in a two-piece muslin cheese bandage and placed in a 20-lb., Wilson-style cheese hoop. In the initial trials the 20-lb. blocks were pressed overnight by stacking the hoops four high,

TABLE 2

Procedure of making rindless Swiss-type cheese in 20-lb. blocks

Step involved	Elapsed time *(hr.: min.)*	Temp. *(° F.)*	Titratable acidity	Probable pH	Remarks
Received in vat	:00	40–50	0.16	6.60 – 6.70	Fat test 2.5-2.6%. S.N.F. 8.5-8.6%.
Starters added	:30	88	0.17	6.50 – 6.60	1.25% lactic culture plus 25 ml. propionic culture/ 1,000 lb. milk. Ripening may be for only 45 min.
Rennet added	1:30	88	0.18	6.40 – 6.50	3 oz/1,000 lb. milk.
Curd cut [a]	1:55	88	0.11	6.35 – 6.45	Use ¼-in. knives and continue cutting until size of tapioca.
Whey drawn off	2:15	88	0.115	6.30 – 6.40	Reduce to ⅔ original volume.
Hot water on	2:35	88	0.12	6.30 – 6.40	145-150° F. water.
Hot water off	2:50	102	0.085–0.09	6.30 – 6.40	From ⅓ to ¼ volume of original milk added.
Stirring complete	3:50–4:05	100	0.11	6.10 – 6.20	Firmness similar to Swiss.
Dam in place	4:00–4:15	100	0.11–0.115	6.10 – 6.20	15-20 lb. wt/block used to press while dam in place. Curd mass approx. 6 in. thick.
Whey drained	4:10–4:25	99	0.13–0.14	5.90 – 6.10	
Dam removed	5:10–5:25	99	0.20–0.25	5.80±0.10	Curd mass cut into blocks 9-10 in. wide and 13 in. long.
Began pressing [b]	5:50–6:10				
Out of press	Next day (after 18-20 hr.)			5.10±0.05	Cheese held at 45° F. one to seven days before placing in warm room at 63-65° F.
Eye formation [c]	2-8 days	63–65			Majority of eyes attain desired diameter of ½ to ¾ in. after 3 to 4 wk.

[a] Slightly softer than for Cheddar Cheese.

[b] Cheese blocks wrapped in two-piece muslin bandage and placed in 20-lb. Wilson-style hoop for pressing. The cheese was pressed 15 min., dressed, pressed for an hour, redressed, and pressed overnight with 10-15 lb/sq in. (hydraulic) or a total of 2,400 lb. (spring press).

[c] After eye development has occurred, the cheese is held at 40° F. until sold.

with a 50-lb. weight placed on top. In subsequent lots, the cheese was pressed overnight in horizontal presses with 10-15 lb/sq in. (hydraulic) or a total of 1,600-2,400 lb. (spring press). The desired pH after pressing was 5.10 ± 0.05. A pH of 5.0 did not seem detrimental, but a pH above 5.20 was associated with too many eyes and too rapid eye development. This is in accord with the recommendations of other investigators relative to the pH desired in Emmanthaler cheese following pressing (5, 6, 14).

A salting period of two days in saturated brine at 45° F. proved most satisfactory, yielding a cheese with a salt content of approximately 1.25% and the most desirable flavor. Cheese salted 24 hr. contained 1.0 to 1.1% salt and was slightly bitter at times. The salt content was increased to above 1.5% by three days of salting, and a slight Cheddar flavor sometimes was found. Eye formation occurred slightly closer to the surface of the cheese with one or two days of salting than with a three-day salting period.

The press cloths were removed after salting and the cheese was held approximately 4 hr. at 45° F. to allow partial drying of the cheese surface. The cheese block then was trimmed, wrapped in a film wrap (Cryovac [4] or Saran [5]) held in place by tape rather than sealed, and then held at 45° F. for a period of one to 14 days to permit more even salt distribution in the block before placing in the warm room for eye development. Although no consistent differences in eye formation were attributed to the variation in length of the holding period, it was felt that cheese held for a four- to seven-day period had somewhat more uniform eye formation than that placed immediately in the curing room. Holding 14 days offered no apparent advantage.

The procedure finally adopted which produced the most desirable cheese is outlined in Table 2. Cured cheese made by this procedure had a composition as follows: 38.5 to 39.5% moisture, 26.50 to 27.00% fat, 43.75 to 44.00% fat on a dry basis, and a salt content ranging from 1.2 to 1.5%. The yield of cured cheese per 100 lb. of cheese milk varied from 8.0 to 8.2 lb. The composition of this cheese was very similar to that reported by Sanders *et al.* (13) for domestic Swiss cheese, except for being slightly lower in fat.

When 3 to 4 mo. of age the cheese had a mild, slightly sweet flavor. Holding 8 to 12 mo. brought a more definite Swiss cheese flavor accompanied in some lots by a slightly bitter flavor.

ACKNOWLEDGMENT

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[4] Manufactured by W. R. Grace and Co., The Cryovac Division, Cambridge 40, Mass.
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RINDLESS, SWISS-TYPE CHEESE. II. PREVENTION OF SURFACE MOLD GROWTH DURING CURING AND STORAGE[1]

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SUMMARY

Methods of preventing mold growth on the surface of rindless Swiss-type cheese, cured in an unsealed film wrap, were investigated. Painting the cheese with a 15 to 20% aqueous solution of potassium sorbate, 1 oz/20-lb. block, resulted in very slight mold growth and small cleaning losses during curing periods up to 9.5 mo. Holding for longer periods allowed slightly more extensive mold growth and cleaning losses. The use of 10% solutions of this compound was less effective. A 1 or 2% solution of sorbic acid in propylene glycol failed to inhibit mold growth sufficiently to be acceptable.

The use of oils or greases was unsatisfactory. Mold growth occurred on all blocks treated with Confecto grease, a candy-slab releasing agent. Mineral oil was inconsistent in its ability to protect the cheese surface against mold growth; at times its use resulted in extensive cleaning losses. The use of Saran film dusted with 2.0 mg. of sorbic acid per 100 sq. in. consistently failed to inhibit mold growth. Cryovac film was preferred slightly over Saran for wrapping the cheese because the former, after shrinking, maintained a closer contact with the cheese surface.

The prevention of surface mold growth on rindless cheese during curing and subsequent storage can be a major problem to a cheese manufacturer. The prevention of such mold growth on rindless block Swiss Cheese by sealing each block in a flexible wrapper has been reported by Stine (17). Gooding (6) reported that certain alpha-beta unsaturated fatty acids, such as sorbic acid (CH_3—CH=CH—CH=CH—COOH) and its salts, are effective fungistatic agents for foods and food wrappers. Sorbic acid–treated cheese wrappers have been found an effective means of preventing surface mold growth in small consumer-sized packages of natural or processed Cheddar Cheese without imparting objectionable flavors (9, 15, 16). Potassium sorbate is another growth inhibitor for molds. When applied to foods at pH 6.5 or lower, this compound hydrolyzes to sorbic acid (12). Melnick *et al.* (11) state that the alpha-beta unsaturated acids, such as sorbic acid, can inhibit the dehydrogenase system of molds, thus inhibiting their growth.

Deuel *et al.* (2, 3) reported that sorbic acid was harmless as a dietary agent and was readily metabolizable to CO_2 and H_2O under normal conditions. Both sorbic acid and the potassium salt were included among those additives found safe and acceptable for use in food by the Food and Drug Administration (4, 5).

In this investigation, the effectiveness of treating the surface of blocks of Iowa Swiss-type cheese with various materials to prevent or restrict mold growth under an unsealed film wrap was studied.

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METHODS AND MATERIALS

The Swiss-type cheese was made according to the method previously reported (1). After a 48-hr. salting period the blocks were removed from the brine, the bandages stripped off, and the blocks allowed to dry 2-4 hr. at 40-45° F. before wrapping in film. The blocks were trimmed carefully to insure close contact between cheese surface and wrapper. Care was taken to minimize contamination of the cheese surface with mold. Those blocks treated before wrapping were painted with approximately 1 oz. of the material tested. The amount used was determined by weighing the block before and after treatment on a scale which read to 0.01 lb. The materials used to treat the surface of the cheese and the films used in wrapping the blocks are listed in Table 1.

TABLE 1

Materials used to treat and wrap cheese

Trade name	Material	Manufacturer or source
	Material used to treat cheese surface before wrapping	
Sorbic	Sorbic Acid	Carbide and Carbon Chemical Co., New York, New York
Sorbistat-K	Potassium sorbate	Chas. Pfizer and Co., Inc., Brooklyn 6, New York
Confecto grease	Emulsion of butyl stearate and hydrogenated vegetable oils	International Foodcraft Corp., Brooklyn 11, New York
Superla white mineral oil No. 21	U.S.P. mineral oil, heavy	Standard Oil Company, Chicago, Illinois
	Film used to wrap cheese	
Saran No. 5, 100-gauge, plain	Vinylidene chloride and vinyl chloride copolymer	Dow chemical Co., Midland, Michigan
Saran No. 5, 100-gauge, dusted with sorbic acid	Vinylidene chloride and vinyl chloride copolymer dusted with sorbic acid [a]	Dow Chemical Co., Midland, Michigan
Cryovac, 75-gauge	Polyvinylidene chloride resin blends	Cryovac Division, W. R. Grace and Co., Cambridge 40, Massachusetts

[a] Dusted with 2.0 mg. sorbic acid/100 sq. in.

The sorbic acid was 94.49% pure with 5.06% water. Because of its low solubility in water (0.2%), solutions of sorbic acid were prepared in reagent-grade propylene glycol (7, 18). The potassium sorbate had a purity, as measured by total potassium content, of 98 to 101% and a moisture content of 1% maximum (12). The Confecto grease is a slab-releasing agent used in the candy industry (14). It is a nonaqueous emulsion of both liquid and solid phases of butyl stearate and hydrogenated vegetable oils of low iodine value containing antioxidants (8). This material begins to soften at 80° F. and is not completely melted at 110° F. It was very difficult to apply smoothly to a cold block of cheese with a brush. U.S.P. mineral oil has been used in the past as a coating

to reduce surface mold growth on a variety of cheeses. This material was used in the study to serve as a basis of comparison, although its use is not legal at present.

Cryovac film is heat-shrinkable due to an orientation of the molecules in the film by a special process (13). Therefore, all blocks wrapped in this film were dipped in hot water at 180 to 190° F. to shrink the film more tightly to the surface of the cheese block. The Saran film, used in the first series of trials, was not heat-shrunk (19). Scotch brand No. 600 [2] cellophane tape was used to hold the film on all blocks in the first series of trials. A pressure-sensitive vinyl plastic tape No. 473 [2] or Paklon brand film tape No. 681 [2] was used to hold the wrap in place in the second series of trials.

After wrapping, the blocks were placed in open-topped wooden boxes and held at 40-45° F. for one to 14 days in the early trials and for two to four days in later trials. The boxes of cheese then were placed in the curing room at 63° F. to bring about eye development. Following eye development, the cheese was held in storage at 35-40° F. until cleaned for packaging for retail distribution.

A mold growth prevention rating (MPR) based on the criteria listed in Table 2 was given each block when placed in storage following eye development and again when removed from storage and cleaned for packaging. In a number of lots the weight loss that occurred in cleaning the individual blocks of cheese also was determined. A MPR rating of seven or below indicates a degree of mold growth unacceptable to a commercial plant because of the cost of cleaning and cleaning losses.

The wrap on some blocks was punctured, due to careless handling, while curing or in storage. In a few instances the wrap become loose due to a failure of the tape used to hold the wrapper in place. Data from those blocks with a punctured or loose wrap were not included in this study.

RESULTS AND DISCUSSION

The effect of the surface treatment and film wrap used in the first series of trials on the development of mold growth during storage and the subsequent cleaning losses is shown in Table 3. The results obtained when the blocks were wrapped without previous treatment indicate the necessity of preventing or minimizing surface mold growth during curing and storage of film-wrapped Iowa Swiss-type cheese. This group had a lower median MPR and a greater cleaning loss than when any of the preparations were used to treat the cheese surface. A MPR of 5, the highest median rating found when no surface treatment was used, was below that considered acceptable to the commercial cheese plant. The majority of the blocks in the first series of trials were wrapped in plain Saran film. Wrapping the untreated blocks with Saran film dusted with 2.0 mg. of sorbic acid per 100 sq. in. offered no consistent improvement over plain Saran with the few blocks on which it was used. This concentration of sorbic acid is considerably lower than the 2.5 to 5.0 g/100 sq. in. used by Smith

[2] Manufactured by the Minnesota Mining Co., St. Paul, Minnesota.

and Rollin (16), which would explain the incomplete inhibition of mold growth. The Cryovac film, which was heat-shrunk to cling close to the cheese surface, did not materially reduce the incidence of mold growth below that found with the two other films.

Treating the cheese with 1 or 2% sorbic acid solutions did reduce mold growth, but not to the extent desired. While some blocks treated with 2% sorbic acid remained free of visible mold growth, others showed objectionable amounts of growth. Mold growth was observed on all five blocks treated with a 1% solution of sorbic acid.

The inhibition of mold growth by mineral oil, although inconsistent, was somewhat greater than that found with a 1% sorbic acid solution and compared favorably with that produced with a 2% sorbic acid solution. The use of Saran film dusted with sorbic acid (2.0 mg/100 sq. in.) following the surface treatment with mineral oil also failed to give adequate protection against surface mold growth. Cryovac film, which was heat-shrunk to cling close to the cheese

TABLE 2

Criteria for determination of mold growth prevention rating

Extent of mold growth				Mold growth prevention rating (MPR)
Very slight, 5 spots	Slight, 10% of area	Moderate, 10-50% of area	Heavy, 50% area	
No surface	No surface	No surface	No surface	10
One surface	No surface	No surface	No surface	9
Two surfaces	One surface	No surface	No surface	8
Three surfaces	One surface + v. sl.[b] on second surface	No surface	No surface	7
Four surfaces	Two surfaces or one sl.[a] + two surfaces with v. sl. growth	One surface	No surface	6
Five surfaces	Two surfaces + v. sl. on third surface	One surface + v. sl. on second surface	No surface	5
All surfaces	Three surfaces or two with sl. and two with v. sl. growth	One surface + sl. on second	One surface	4
All surfaces	Three surfaces + v. sl. on one	Two surfaces	One surface + sl. on one	3
All surfaces	Four surfaces	Two surfaces + sl. on third surface	One surface + two with sl. growth or one with mod.[c] growth	2
All surfaces	Four or more surfaces	Three or more surfaces	Two or more surfaces	1

[a] sl. = slight.
[b] v. sl. = very slight.
[c] mod. = moderate.

TABLE 3

Effect of surface treatment and film wrap on mold growth and resulting cleaning losses of experimental lots of block style, rindless Swiss-type cheese

Surface treatment prior to wrapping	Film wrap	No. of blocks	Total time wrapped	Storage time		Mold growth prevention rating (MPR)			Cleaning loss		
				Eye room	Cold room	Range	Mean	Median	Range	Mean	Median
			(mo.)	*(wk.)*	*(wk.)*				*(%)*	*(%)*	*(%)*
None	Saran	25	4	2–4	4–13	1– 6	3.2	3.0	0.18–3.49	0.97	0.72
		17	4–6½	1½–4	17–24	1– 8	4.5	4.0	0.05–1.08	0.54	0.55
		6	6½	4–6	26–27	1– 7	2.7	1.5	0.82–1.92	1.31	1.57
	Saran + S.A. dust [a]	3	4	4	8	2– 4	3.0	3.0	1.33–2.31	1.81	1.80
	Cryovac	2	4	4	8	4	4.0	4.0	0.61–1.60	1.11	1.11
		7	4–6½	1½–2	18–21	3– 8	5.3	5.0	0.09–1.04	0.43	0.48
Propylene glycol	Saran	5	4	2–3	5–12	2– 6	4.8	5.0	0.22–1.45	0.73	0.51
1% Sorbic Acid in propylene glycol	Saran	5	4	2–3	4–13	4– 8	5.6	6.0	0.36–0.52	0.41	0.40
2% Sorbic Acid in propylene glycol	Saran	5	4	2–3	4–13	4– 6	5.6	6.0	0.17–0.58	0.36	0.35
		5	4–6½	1½–3	21–24	4–10	8.0	8.0	0.00–0.46	0.14	0.05
	Cryovac	2	4–6½	1½	21	10	10.0	10.0	0.00	0.00	0.00
Mineral oil	Saran	6	4	2–4	5–12	1–10	5.3	5.0	0.00–1.61	0.56	0.27
		5	4–6½	1½–2	18–24	4–10	7.6	8.0	0.00–0.18	0.09	0.09
	Saran + S.A. dust [a]	3	4	4	8	6– 8	7.0	7.0	0.05–0.32	0.19	0.21
	Cryovac	2	4	4	8	8–10	9.0	9.0	0.00–0.05	0.03	0.03
		5	4–6½	1½–2	18–21	6–10	7.4	7.0	0.00–0.27	0.13	0.10

[a] Saran dusted with 2.0 mg. sorbic acid/100 sq. in.

surface, was somewhat more effective in helping to minimize mold growth on the treated blocks, since it made possible a closer contact of film, the material used to treat the cheese surface, and the cheese surface.

Scotch-brand cellophane tape was not satisfactory for use with a film wrap shrunk with hot water. The adhesive failed to hold at dipping and the tape had to be reapplied or, in some instances, the block had to be rewrapped.

The relative effectiveness of Confecto grease, mineral oil, and potassium sorbate as inhibitors of surface mold growth was studied in a second series of trials. Each block, after treatment with one of the above materials, was wrapped in Cryovac film and dipped in hot water (180-190° F.) to shrink the wrap. The film was held in place by pressure-sensitive tape, either No. 473 or No. 681.

The mold growth prevention ratings given these blocks of cheese when they were removed from the eye room, and again when brought from storage to be cleaned, are shown in Table 4. The cleaning losses also are shown in Table 4.

Confecto grease proved to be the least effective mold inhibitor of the materials tested in this series of trials. The Confecto grease was difficult to apply to the cheese block and considerable mold growth occurred during eye formation and subsequent storage. Mineral oil, while more effective than Confecto grease, again was inconsistent in affording adequate protection against mold growth.

Potassium sorbate, in proper concentration, proved satisfactory. One ounce of 20% potassium sorbate painted on the entire surface of the 20-lb. cheese block allowed little or no mold growth during storage up to 9 mo., with subsequent cleaning losses of less than 0.10%. When stored for longer periods, the fungistatic properties were good, although the cleaning losses were slightly higher. No off-flavors were attributable to potassium sorbate. A 15% solution of potassium sorbate was equally effective as a 20% solution for storage periods of 6 mo. or less, and nearly as satisfactory for storage periods up to 1 yr. A 10% solution did not give adequate protection.

In the second series of trials a direct relationship was observed between the MPR of the blocks with intact wrappers and the cleaning loss. A total of 294 blocks was examined. Of these, 138 received a MPR of ten and, although no mold growth was observed, sustained cleaning losses ranged from 0 to 0.81%, with mean and median losses of 0.17 and 0.10%, respectively. Sixteen blocks were given a MPR of nine. The cleaning loss on this group varied from 0.04 to 0.91%, with mean and median losses of 0.25 and 0.24%, respectively. The cleaning loss on the 34 blocks which received a MPR of eight ranged from 0.04 to 1.43%, with mean and median losses of 0.44 and 0.36%. Approximately one-third of the blocks (106) received a MPR of seven or lower. The cleaning loss on the 16 blocks with a MPR of one, the lowest rating given, ranged from 1.60 to 7.12% and averaged 3.25%. Each block of cheese, even those with no visible mold growth, usually had to be trimmed slightly when cleaned and cut into consumer portions.

The growth of mold on the surface of cheese blocks treated with sorbic acid or potassium sorbate probably occurred because of a reduced concentration of the fungistatic agent. Melnick and Luckmann (10) have reported that the

TABLE 4

Effect of length of storage on mold growth and cleaning losses of block style, rindless Swiss-type cheese treated and wrapped in Cryovac film

Surface treatment prior to wrapping	Out of warm room							Out of storage								
	No. of blocks	Total time wrapped	No. of blocks	Storage time	Mold growth prevention rating (MPR)			No. of blocks	Storage time	Mold growth prevention rating (MPR)			Cleaning loss			No. of lots
					Range	Mean	Median			Range	Mean	Median	Range	Mean	Median	
		(mo.)		*(wk.)*					*(mo.)*				*(%)*	*(%)*	*(%)*	
Confecto	27	6¾–9½	27	3–5	3–9	6.0	6.0	21	6¾–8½	1–8	3.7	3.0	0.74–4.45	1.81	1.60	3
grease	19	>9½	19	3–4	1–9	6.3	6.0	15	12	1–4	2.0	2.0	0.89–7.12	3.27	2.68	2
Mineral oil	46	4–6½	46	3–6	6–10	8.7	8.5	36	3¼–5¾	3–10	7.6	8.0	0.00–2.21	0.41	0.27	5
	18	>9½	18	5	6–10	8.6	9.0	17	9¾	1–8	4.0	4.5	0.99–4.79	1.97	2.17	1
10% Potassium sorbate solution	17	4–6½	17	4	3–10	7.6	8.0	17	3½	1–9	5.8	6.0	0.04–2.21	0.54	0.35	1
	39	6¾–9½	39	2–5	1–10	9.1	10.0	38	6¾–7	1–10	6.6	7.0	0.05–3.03	0.94	0.65	3
	30	>9½	30	3	9–10	9.6	10.0	25	8½–10¾	3–10	7.6	10.0	0.05–1.28	0.36	0.23	2
15% Potassium sorbate solution	18	4–6½	18	5	6–10	9.2	10.0	18	3	3–10	7.6	9.5	0.00–0.65	0.15	0.05	1
	30	6¾–9½	30	4–6	10	10.0	10.0	29	7¼–8	8–10	9.9	10.0	0.00–0.31	0.13	0.10	3
	18	>9½	18	3	6–10	9.7	10.0	17	10	6–10	9.4	10.0	0.09–1.36	0.47	0.40	1
20% Potassium sorbate solution	18	4–6½	18	6	5–10	9.5	10.0	18	3	3–10	9.3	10.0	0.00–0.69	0.10	0.05	1
	28	6¾–9½	28	4–6	10	10.0	10.0	26	7¼–8	10	10.0	10.0	0.04–0.38	0.11	0.09	3
	20	>9½	20	3	10	10.0	10.0	17	9¾	9–10	9.9	10.0	0.05–0.81	0.29	0.31	1

concentration of sorbic acid on the surface of cheese wrapped in a sorbic acid–dusted wrap decreased to a low level, due to migration of the sorbic acid to the center of the package. Melnick *et al.* (11) also state that molds are capable of metabolizing sorbic acid under conditions where there is a high ratio of mold to sorbic acid, thus reducing the concentration of mold inhibitor. Depending upon the size of the block, the amount of sorbic acid or potassium sorbate originally on the surface, and the length of storage period, the concentration of the mold inhibitor on the surface may be reduced to a point where recontamination results in appreciable mold growth.

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A HIGH-ROUGHAGE SYSTEM FOR RAISING CALVES BASED ON THE EARLY DEVELOPMENT OF RUMEN FUNCTION. X. WHOLE BLOOD, PLASMA, AND CORPUSCLE GLUCOSE RELATIONSHIPS IN CALVES FED HIGH-ROUGHAGE RATIONS WITH AND WITHOUT CHLORTETRACYCLINE

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SUMMARY

In the first of two experiments, the changes in the blood glucose (reducing sugar) of five high roughage–fed calves was studied to 12 wk. of age. In the second experiment, 20 calves fed high-roughage pellets with or without chlortetracycline were compared from birth to 16 wk. of age. Much of the decline in the blood glucose of all calves during the 7-wk. milk feeding period was the result of decreased corpuscle glucose. Plasma glucose was found to decline markedly after milk intake was reduced at 6 wk. Chlortetracycline-fed calves maintained a higher whole blood glucose level. This higher blood glucose level was shown to be largely the result of increased plasma glucose which persisted following termination of milk feeding until 12 wk. of age, when chlortetracycline was withdrawn from the ration. At this point, the plasma glucose of the experimental group returned to the level of the controls. The data lend further support to the postulate that improved calf growth resulting from chlortetracycline feeding is due to its energy-sparing effects, reflected in the plasma glucose level.

In previous experiments involving high roughage–fed calves the blood glucose level was shown to decline markedly during the 7-wk. milk feding period; whereupon, it leveled out at approximately the level maintained by adult ruminants, 40 to 60 mg. % (2).

Earlier, McCandless and Dye (6) had shown that a decline in blood glucose was characteristic of young ruminants and suggested that this represented the change from primary dependence on abomasal digestion to dependence on ruminal digestion for energy. Reid (10), working with lambs, showed that most of the decline during the first 4 wk. was the result of decreased corpuscle glucose and was not considered to be associated with rumen development. In a preliminary report including part of the data in this paper the same observation was made with calves (14). Ratcliff *et al.* (9) later observed that the decline in blood glucose also occurred in calves fed whole milk and a gruel-type replacer diet. Staubus *et al.* (12) observed a less marked drop in calves fed a starter free-choice.

Chlortetracycline (aureomycin) has been reported to be a growth stimulant for calves by many investigators (4). In earlier work we observed that both increased rate of growth and a higher blood glucose level was maintained, even after milk feeding was terminated, when chlortetracycline was included in the

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ration of high roughage–fed calves (2). Voelker *et al.* (15) also showed that the blood glucose level of calves fed antibiotics was higher than their controls following oral administration of glucose, and that calves with higher blood glucose levels gained more in body weight.

It was noted (3) that the proportion of volatile fatty acids in the rumen juice was shifted, butyric acid being higher and propionic acid lower in the aureomycin-fed calves compared to the controls. On the basis of these observations, it was postulated that chlortetracycline increased growth through conservation of energy resulting from deterred fermentation associated with depressed action of certain rumen microorganisms. This seemed likely because certain typical rumen microorganisms which were present in the cud material from older cows became established in the control calves but did not become established in the rumens of the chlortetracycline-fed calves (3).

In this study the changes in plasma- and corpuscle glucose (reducing sugar) were studied from birth to 12 or 16 wk. of age in calves fed a 2:1 mixture of coarsely ground hay and grain or a similar pelleted high-roughage ration (1, 2) with and without chlortetracycline. This was done in an effort to elucidate which blood fractions were affected by the change-over from milk feeding to a high-roughage ration and by feeding chlortetracycline.

Growth, feed consumption, digestibility, and rumen function data obtained in conjunction with these experiments were reported previously (1, 2).

EXPERIMENTAL PROCEDURES AND RESULTS

In two experiments, 25 calves were used to study the changes in blood glucose. All calves nursed their dams for approximately three days and then were fed whole milk at the rate of 1 lb. per 10 lb. of birth weight to 6 wk. of age; the amount of milk fed was gradually reduced to zero during the 7th wk. The ground or pelleted high-roughage rations were offered free-choice after the third day and, from the end of the milk feeding period, constituted the only feed. Wheat straw bedding was used. Water, salt, and bonemeal were available free-choice.

Weekly blood samples were drawn from the jugular vein at approximately 3 hr. after the morning feeding. Whole blood and plasma glucose were determined immediately by the method of Somogyi (11), using Nelson's reagent (8). Red cell volume and plasma volume were determined on separate aliquots by centrifuging for 30 min. at 2,000 × G. The glucose values of various blood fractions were expressed in two ways: (a) the partition of the total glucose in 100 ml. of whole blood into the amount in the plasma (mg/100 ml. of whole blood) and the amount in the corpuscles (mg/100 ml. of whole blood) and (b) the concentration of glucose expressed as mg/100 ml. in each of these two fractions. In order to accomplish this, analyses were made for whole blood glucose, plasma glucose, packed red cell volume, and plasma volume. Milligrams of plasma glucose (mg/100 ml. of whole blood), corpuscle glucose (mg/100 ml. of whole blood), and corpuscle glucose (mg/100 ml.) were then calculated using the following formulae:

$$\text{Plasma glucose (mg/100 ml. whole blood)} = \frac{\text{Plasma vol. (\%)} \times \text{Plasma glucose (mg/100 ml.)}}{100}$$

Corpuscle glucose (mg/100 ml. whole blood) =
Whole blood glucose (mg/100 ml.) − Plasma glucose (mg/100 ml. whole blood)
Corpuscle glucose (mg/100 ml.) =

$$\frac{100 \times \text{Corpuscle glucose (mg/100 ml. whole blood)}}{\text{Red cell volume (\%)}}$$

Experiment 1. This experiment was conducted to study the changes in plasma and corpuscle glucose in calves during and after the 7-wk. milk feeding period to 12 wk. Five calves, four Holsteins and one Jersey, were fed whole milk to 7 wk. of age and from the third day to 12 wk. they were offered, free-choice, a high-roughage mixture composed of two parts coarsely ground, third-cutting alfalfa and one part simple grain mixture. The formula and composition of this ration are shown in Table 1. Changes in the concentration of plasma, corpuscle, and whole blood glucose are shown in Figures 1 and 2.

TABLE 1

Composition of high-roughage rations

Ration group	Experiment 1	Experiment 2	
	High-roughage mixture	Pellets A	Pellets B
Ground alfalfa hay *(%)*	66.6	66.0	65.54
Ground shelled corn *(%)*	14.7	25.2	25.1
Ground whole oats *(%)*	11.0	.....	.....
Wheat bran *(%)*	3.7	.....	.....
Soybean oil meal *(%)*	3.7	8.5	8.5
Salt *(%)*	0.3	0.3	0.3
Aurofac 2-A *(%)*[a]	.....	.....	0.56
	100.0	100.0	100.00

[a] Contains 3.6 g. chlortetracycline hydrochloride (aureomycin) per pound.

Experiment 2. In this experiment, 20 Jersey calves were divided into two groups and used to study the influence of chlortetracycline on the whole blood, plasma, and corpuscle glucose to 16 wk. of age. Half the calves in each group were inoculated with fresh cud material (2). Starting at three days of age and continuing to 16 wk., high-roughage pellets (a) composed of two parts ground alfalfa hay and one part simple grain mixture were fed free-choice to the control group. Chlortetracycline (Aurofac 2-A) was added at the rate of 20 mg/lb as a micro-ingredient in the pellets (b) fed the experimental group to 12 wk. of age. From 12 to 16 wk. these calves were also fed pellets (a) containing no chlortetracycline. The composition of the rations used is shown in Table 1. The results of the blood analyses in this experiment are presented in Figures 3 and 4. No differences in the blood picture were found between inoculated and uninoculated calves; thus, the data for each pellet group, (a) and (b), were combined.

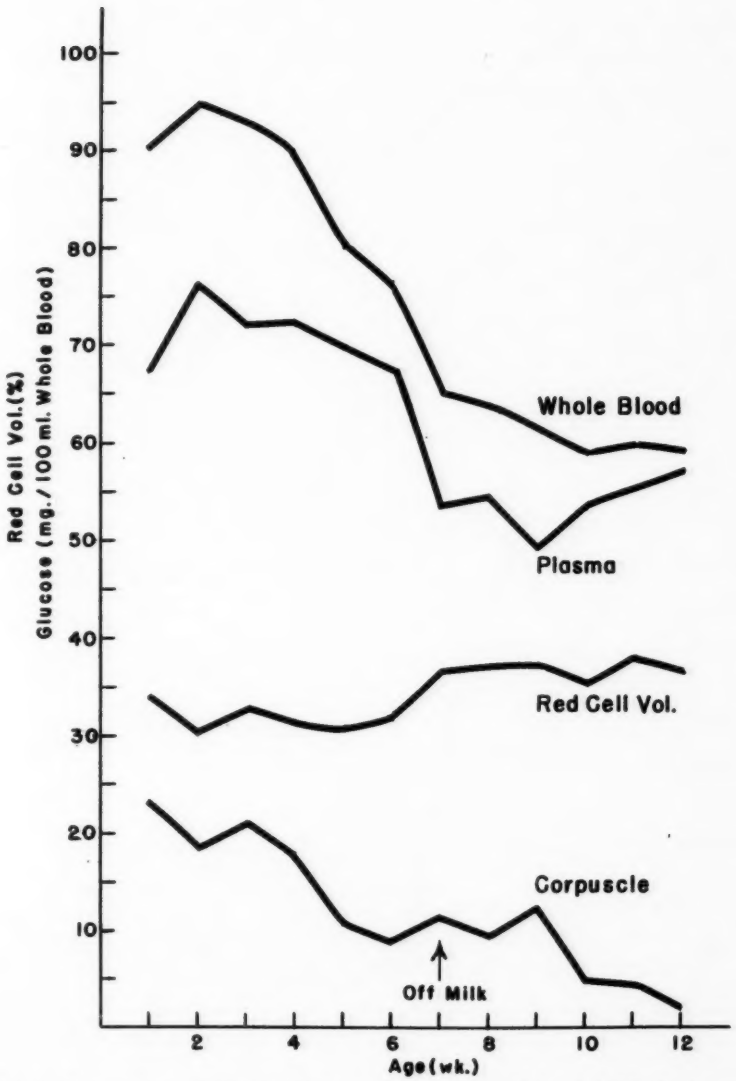

FIG. 1. Changes in concentration of glucose (reducing sugar) in whole blood, plasma, and corpuscles (mg/100 ml. of whole blood) and red cell volume (per cent) in calves fed a high-roughage ration. Experiment 1.

DISCUSSION OF RESULTS

These data confirm previous reports (9, 10, 14) that much of the decline in the blood glucose of young calves during the first 7 wk. is the result of a precipitous decline in corpuscle glucose (Figures 1, 2, 3, and 4). The lowered

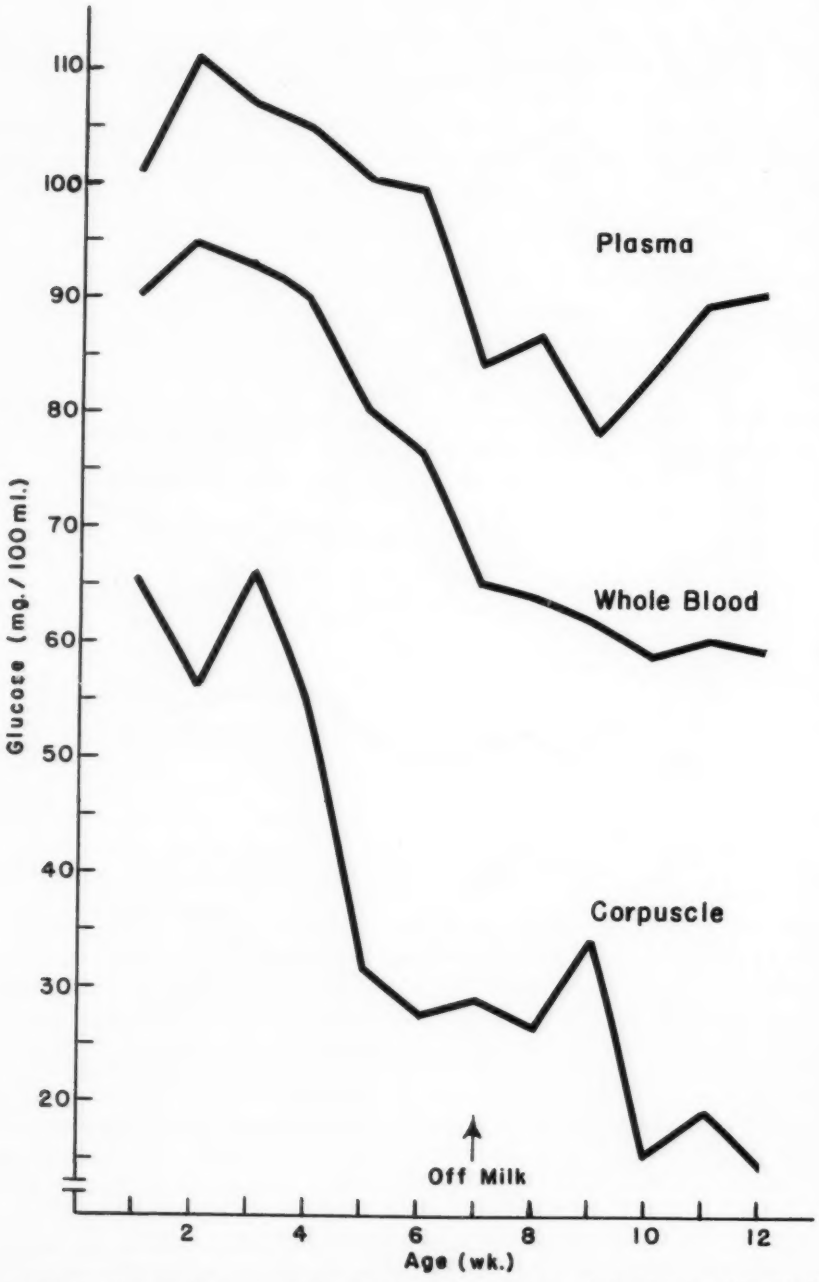

FIG. 2. Changes in concentration of glucose (reducing sugar) in plasma, whole blood, and corpuscles (mg/100 ml.) in calves fed a high-roughage ration. Experiment 1.

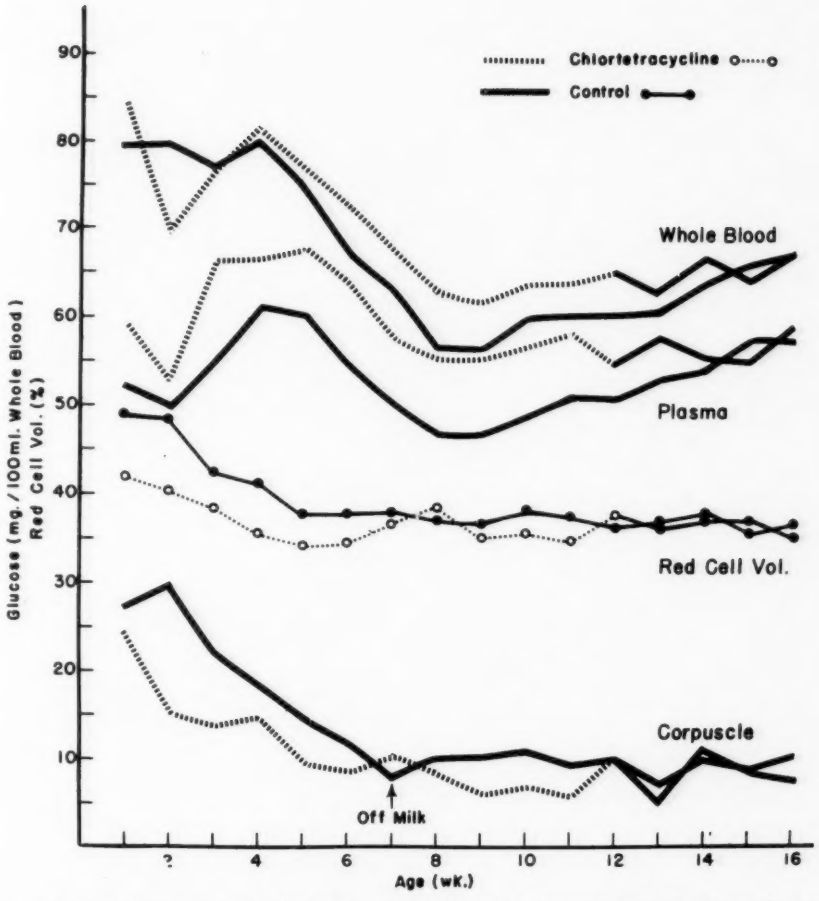

FIG. 3. Influence of chlortetracycline on the changes in concentration of glucose (reducing sugar) in whole blood, plasma and corpuscles (mg/100 ml. of whole blood) and red cell volume (per cent) in calves to 16 wk. of age. Experiment 2.

corpuscle glucose appeared to be the result of loss of fetal red cells and replacement with postnatal red cells in which the concentration of glucose was lower (13).

Plasma glucose also declined during the 7-wk. milk feeding period, but the sharpest drop occurred upon withdrawal of milk from the ration (Figures 1, 2, 3, and 4). It is interesting to note that plasma glucose levels for the calves shown in Figures 1 and 2 and the control groups in Figures 3 and 4 were lowest just after milk feeding was terminated. From this point they were shown to increase about 14 mg. % by the end of the feeding period. The low point and gradual rise in plasma glucose suggest that the calves were under-

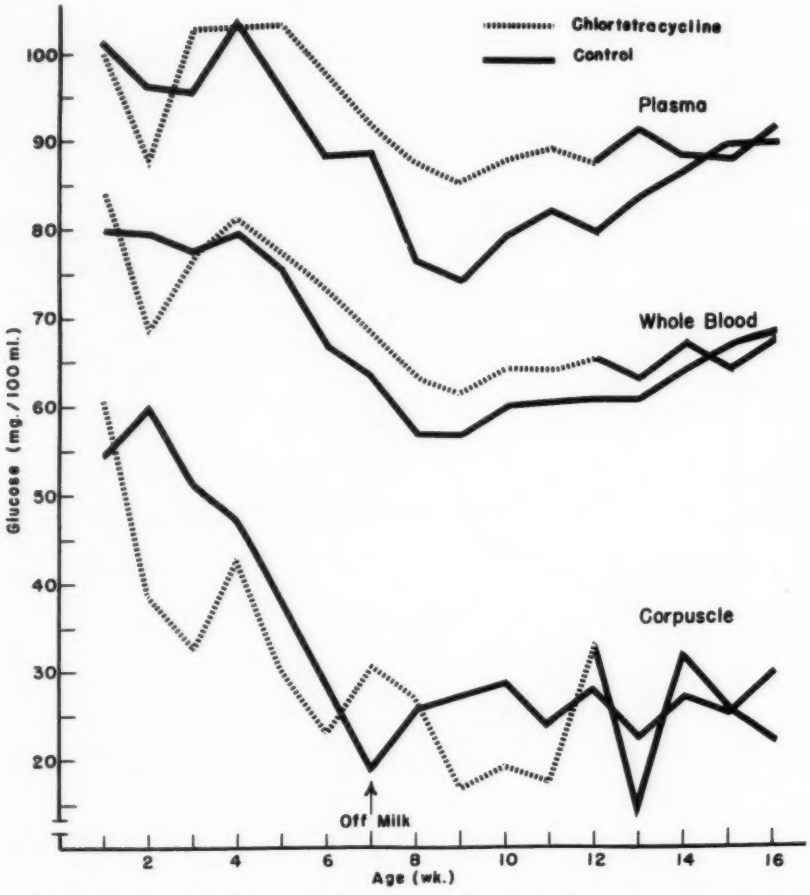

FIG. 4. Influence of chlortetracycline on the changes in the concentration of glucose (reducing sugar) in plasma, whole blood, and corpuscles (mg/100 ml.) in calves to 16 wk. of age. Experiment 2.

going a substantial adjustment in energy metabolism after being changed to complete dependence on the high-roughage rations and probably represent a period of suboptimum energy availability. However, since no serious break occurred in the growth rate of the calves at this point (2), the depression in plasma glucose at weaning may not be a critical problem.

As previously observed (3), chlortetracycline-fed calves maintained higher whole blood glucose levels ($P < 0.01$). These higher blood glucose levels were shown to be largely the result of increased plasma glucose (Figures 3 and 4). An analysis of variance of the means during the 8- to 12-wk. period, following removal of milk from the ration, revealed that the plasma glucose values, both

on the basis of mg/100 ml. of whole blood and mg/100 ml. of plasma, were significantly higher than the controls ($P < 0.01$). In the period after the chlortetracycline was removed from the ration, 12-16 wk., there were no significant differences between the groups. It is noteworthy that chlortetracycline prevented the depression of plasma glucose observed in the control calves following withdrawal of milk from the diet (Figures 3 and 4).

These data strongly support the postulate that the influence on calf performance resulting from feeding low levels of chlortetracycline arises from its energy-sparing effects, reflected in the plasma glucose level. Other data published by the authors (3) suggest that this may result from the conservation of glucogenic substances as they pass through the rumen, associated with a shift in volatile fatty acid concentration in the rumen.

It was shown previously (3, 5) that sudden withdrawal of chlortetracycline from the ration of calves resulted in decreased rate of growth and decreased efficiency of feed utilization. Rumen inoculations administered after aureomycin feeding was terminated improved calf performance (7). These observations, considered together with the observation that these effects were not found when the rumen was by-passed by feeding chlortetracycline in the milk (2), also implicate the rumen as the primary site of action of chlortetracycline on the energy metabolism of high roughage–fed calves.

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FATTY ACID COMPOSITION OF THE LIPID FRACTIONS FROM BOVINE SERUM LIPOPROTEINS[1, 2]

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SUMMARY

Weights and fatty acid composition of the lipid fractions have been determined from bovine serum lipoproteins separated ultracentrifugally at a solvent density of 1.063 g/ml and corresponding in electrophoretic mobility to α- and β-lipoproteins. The vast proportion of serum lipids was contained in the high-density lipoproteins ($D > 1.063$), with the marked exception of the triglycerides, which were evenly divided. Degree of saturation in the high-density lipoprotein total lipids was 27% and ranged from cholesteryl esters 8.4%, phospholipids 50%, nonesterified fatty acids 65%, to triglycerides 80%. The low-density lipoproteins ($D < 1.063$) showed a 74% degree of saturation in their total lipids which varied from phospholipids 73%, triglycerides 84%, nonesterified fatty acids 86% to cholesteryl esters 89%. Fatty acid distribution indicated a large concentration of linoleate in the total lipids and cholesteryl esters of the high-density lipoproteins. This concentration was dispersed in their other lipid fractions. The low-density lipoproteins contained chiefly palmitate and stearate in all lipid fractions. Triglycerides in both high- and low-density lipoproteins were notably similar in weight, saturation, and fatty acid composition.

During the past decade serum lipids have been studied from many perspectives. In the bovine, among other metabolic aspects an importance as milk fat precursors has been demonstrated (8, 21). Studies of bovine serum lipids, however, have dealt only with whole serum (3, 6, 8, 12, 14, 15, 21). In the area of human physiology Cohn (1) and Oncley *et al.* (19) showed that practically all of the plasma lipids are transported by plasma proteins in physical-chemical complexes. Characteristic changes in the concentration of certain of these lipoprotein complexes were found to occur in human diseases such as nephrosis, idiopathic hyperlipemia, infectious hepatitis, and primary biliary cirrhosis (9). A group of cooperating investigators (22) evaluated measurements of the lipoproteins and agreed that those of density lower than 1.063 g/ml were associated with clinical manifestations of atherosclerosis. Since then many studies have been conducted in an effort to delineate significant differences between the lipoprotein classes. Many physical properties and chemical characteristics have been described. Among these investigations, Nelson and Freeman (17) outlined the phospholipid and phospholipid fatty acid content of certain human serum lipoproteins. However, no studies have as yet analyzed the fatty acid composition of all the classic lipid fractions contained in serum lipoproteins.

In an effort to throw additional light on the problem of bovine fat metabolism, as well as on the larger and perhaps related problem of human athero-

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sclerosis, a study of the fatty acids in the lipid fractions from bovine serum lipoproteins was undertaken.

Human serum lipoproteins can be divided into variously titled, but corresponding classes depending on the isolation procedure involved (1, 2, 13). Ultracentrifugation of serum adjusted to a density of 1.063 g/ml separates them into two main density groups. One is designated as high-density ($D > 1.063$) and sediments, along with the serum proteins, toward the bottom of the tube, whereas the other is designated as low-density ($D < 1.063$) and floats to the top as a concentrated opaque layer. These large fractions correspond in solubility to lipoproteins obtained by ethanol-water precipitation in Classes IV, V, and VI and I and III, respectively (9). They further correspond in electrophoretic mobility to those that are separated as α- and β-lipoproteins (9). The high-density lipoproteins migrate with the α-globulins and the low-density lipoproteins with the β-globulins. Similar migration patterning has been observed in electrophoretic studies of serum lipoproteins from oxen and calves (16, 23). Because of marked differences in composition and concentration between the high- and low-density lipoproteins in normal as well as pathological conditions (9), this division seemed fruitful for analysis in the bovine.

METHODS

Blood was drawn from the jugular vein of four normal mature Holstein cows representing various stages of lactation. They were on a diet of blue grass pasture supplemented with mixed hay and a common 16% protein grain mixture. After being allowed to clot for 2 hr. at room temperature, the blood samples were centrifuged at 552 × G for 45 min. and the serum separated. Two main lipoprotein groups were obtained by ultracentrifugation of the serum adjusted to a solvent density of 1.063 g/ml (9), followed by quick freezing and tube-sawing (5). The high-density lipoproteins were separated, together with the serum proteins, in the bottom portion of the ultracentrifuge tubes, while the low-density lipoproteins were isolated in an opaque layer at the top. An electrophoretic study was made of the separated lipoprotein groups to ascertain their correspondence in mobility to α- and β-lipoproteins. A horizontal-flow electrophoresis apparatus was used, with a technique essentially similar to that of Jencks (11). After staining for lipid and protein (11), a comparison was made between the lipoprotein migration distances and those of the α- and β-globulins. This procedure also demonstrated that the ultracentrifugally separated lipoproteins were not contaminated with one another. Total lipids were then extracted as reported earlier (15) from the high-density lipoproteins, low-density lipoproteins, and 100 ml. of serum which they represented. Extracts were evaporated at 40° C. under water pump vacuum. Weights of these extracts were obtained from the last two cows studied. Lipid fractions were isolated on silicic acid columns (10) and their identity ascertained by infra-red spectroscopy. After being weighed (in the case of the last two cows), the lipid fractions were subjected to transmethylation, to convert their constituent fatty acids to methyl esters. This was accomplished using a solution of 1% H_2SO_4 in methanol and

refluxing for 2 hr. Methyl esters were separated by gas chromatography, using Barber-Colman Model 10 and Model 20 instruments. The stationary phase for the chromatography columns was ethylene glycol succinate polyester coated on Celite, 80-100 mesh. The column length was 9 ft., column temperature was 180° C., and the carrier gas was argon at 20-lb. pressure. Duplicated runs at these operating conditions showed a measurement error within 1% (4). Identification and quantitation procedures have been previously described (20).

A confirmatory study of 12 normal Holstein cows was made regarding the fatty acid distribution in their total serum lipids. Techniques used for lipid extraction and transmethylation were similar to those of the main study.

RESULTS AND DISCUSSION

The terms high- and low-density lipoproteins are used in this discussion to designate the bovine serum lipoproteins which separate ultracentrifugally at a solvent density of 1.063 g/ml and migrate with the α- and β-globulins, respectively, when subjected to electrophoresis. Included as high-density lipoproteins are all lipoproteins of density greater than 1.063 g/ml. Lipoproteins of density less than 1.063 g/ml, together with any chylomycra which may be present in normal bovine serum, are included as low-density lipoproteins. The basic solvent density condition of 1.063 g/ml was considered worth using in this study as a basis for comparison with other studies of human and animal serum lipoproteins that had been separated at that same density (2, 5, 9, 17). This decision was reinforced by finding that the bovine serum high- and low-density lipoproteins separated in this manner showed electrophoretic mobility of α- and β-lipoproteins, respectively.

Weights of the various lipid fractions, obtained from the last two cows used in this study, are presented in Table 1. It can be seen that the high-density lipoproteins contain the preponderant part of the serum lipids with the notable exception of the triglycerides. Havel *et al.* (9) have shown the tendency of lipids to be concentrated in the serum high-density lipoproteins of other animals as well, but to not such an appreciable degree. Human serum has been demonstrated by Oncley (18) to contain an increased proportion of only phospholipids in its high-density lipoprotein fraction, whereas the other lipids, especially triglycerides, were greater in the low-density lipoproteins. Weights of the whole serum lipid fractions are in essential agreement with those of Lough *et al.* (14).

Table 2 is based on mean values from four Holstein cows. It shows the degree of saturation and fatty acid distribution among the lipid fractions from whole serum and from high- and low-density lipoproteins. Relative order of saturation between the whole serum lipid fractions, as well as their fatty acid pattern, agrees with those of earlier investigators (6, 12). The degree of saturation, however, disagrees with those studies (6, 12), in that it is lower in the whole serum total lipids and cholesteryl esters while being higher in the triglycerides, nonesterified fatty acids, and phospholipids. These differences in degree of saturation may reflect variations between individual bovine, breeds, sexes,

TABLE 1

Weights and weight percentages of lipid fractions from whole serum and serum lipoproteins in the Holstein

	Cow 1						Cow 2					
	Serum		$D > 1.063$[a]		$D < 1.063$[a]		Serum		$D > 1.063$[a]		$D < 1.063$[a]	
	(wt.)[b]	*(%)*	*(wt.)*[b]	*(%)*	*(wt.)*[b]	*(%)*	*(wt.)*[b]	*(%)*	*(wt.)*[b]	*(%)*	*(wt.)*[b]	*(%)*
Cholesterol esters	231	54	234	56	7.5	33	257	54	264	55	11	37
Triglycerides	12	3	6.1	1	6.3	27	13	3	7	2	7.3	24
Nonesterified fatty acids	8.9	2	7.8	2	1.1	4	9.3	2	8.1	2	1.3	4
Cholesterol	30	7	29	7	1	4	30	6	29	6	1	4
Phospholipids	148	34	141	34	7.4	32	166	35	169	35	9.3	31
Total	430	100	418	100	23	100	475	100	477	100	30	100

[a] $D > 1.063$ refers to high-density lipoproteins; $D < 1.063$ refers to low-density lipoproteins.
[b] Milligrams per 100 ml. of serum.

TABLE 2

Fatty acid composition[a] and per cent saturation of lipid fractions from whole serum and serum lipoproteins in the Holstein

Fatty acid	Total			Cholesteryl esters			Triglycerides			Nonesterified fatty acids			Phospholipids		
	Serum	D > 1.063[b]	D < 1.063[b]	Serum	D > 1.063	D < 1.063	Serum	D > 1.063	D < 1.063	Serum	D > 1.063	D < 1.063	Serum	D > 1.063	D < 1.063
C_8	.3	.....[c]	.....	.....	.....	.....	.3	.1	.5	.....	.....	.....	.1	.1	.3
C_{10}	.3	.4	.2	.3	.3	.6	.5	.5	.3	.5	.3	1.9	.1	.2	.6
C_{12}	.2	.2	.6	.3	.1	1.3	.8	.5	.8	.6	.5	2.9	.2	.3	1.0
C_{14}	1.4	1.1	1.7	2.5	1.3	4.6	2.4	2.2	2.5	1.6	1.6	7.0	1.0	.6	1.2
$C_{14}^{=}$	.5	.4	.7	1.0	.9	1.4	1.0	1.0	1.0	.2	.2	.7	.2	.4	.....
C_{16}	13	12	27	7.0	5.9	30	27	27	27	24	23	39	22	18	26
$C_{16}^{=}$	2.7	3.0	2.5	2.6	2.4	3.8	3.0	3.4	2.8	4.5	4.5	3.4	2.4	2.8	2.8
C_{18}	18	13	44	1.9	.8	52	51	50	53	37	38	35	40	31	44
$C_{18}^{=}$	7.8	7.5	12	3.0	3.4	3.8	14	15	12	28	28	10	14	20	11
$C_{18}^{2=}$	49	54	9.1	72	76	1.2	.....	.....	.....	4.0	4.1	.....	20	27	13
$C_{18}^{3=}$	6.6	8.0	2.0	9.0	9.1	1.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
	Per cent saturation[d]														
	33	27	74	12	8.4	89	82	80	84	64	65	86	63	50	73

[a] Expressed as mean weight per cent of the total fatty acids.
[b] D > 1.063 refers to high-density lipoproteins; D < 1.063 refers to low-density lipoproteins.
[c] Less than 0.1%.
[d] Obtained by summing weight percentages of the saturated fatty acids.

feeding regimes, and/or experimental procedures. A greater awareness of possible interbreed and sex variances seems especially worth-while, considering the contemporary lack of information in these areas.

A great difference in degree of saturation can be seen between the total lipid fatty acids of the high- and low-density lipoproteins. The high-density lipoproteins show a very slight total saturation that varies from a low level in the cholesteryl esters through a medium range in phospholipids and nonesterified fatty acids to a much greater degree of saturation in the triglycerides. The low-density lipoproteins, however, evince an elevated total saturation which prevails throughout their lipid fractions. The variance in total saturation is different from that found, thus far, in lipoproteins of human serum. Gillies *et al.* (7) have demonstrated fractions, corresponding to the low-density lipoproteins, that contain a much greater proportion of polyunsaturated fatty acids.

A comparison of fatty acid distribution between the lipoprotein lipid fractions indicates a primary concentration of linoleate in the total lipids and cholesteryl esters of the high-density lipoproteins. This effect becomes dispersed in their other lipid fractions, following a course inversely proportional to degree of saturation. The low-density lipoproteins show a consistent concentrating of palmitate and stearate throughout their lipid fractions. However, in the nonesterified fatty acids, a slight decrease in stearate proportion appears compensated by a myristate increase.

The similarity in fatty acid distribution and degree of saturation between the high- and low-density lipoprotein triglycerides appears particularly cogent in connection with their similarity in absolute weight per unit of serum. Since the high-density lipoproteins are much smaller in size than the low-density lipoproteins, and show much less saturation, it is possible that some homeostatic factor might be effecting a dilution of saturated triglyceride concentrations in the low-density lipoproteins by breaking them down to lipoproteins of high density and adapting them with unsaturated cholesteryl esters. This may facilitate transport and insure proper dispersion of the triglycerides. The dilution effect would seem to be physiologically advantageous, inasmuch as increased proportions of low-density lipoproteins have been found closely associated with atherosclerosis and other human diseases.

TABLE 3

Fatty acid composition of total serum lipids in the Holstein (expressed as mean weight per cent and range)

Fatty acid	Weight per cent	Range
C_{12}	.2	.1– .4
C_{14}	1.6	1.2– 2.4
$C_{14}^{=}$	.5	.2– .8
C_{16}	15	9.6–22
$C_{16}^{=}$	4.2	3.2– 5.2
C_{18}	19	9.1–25
$C_{18}^{=}$	12	7.7–17
$C_{18}^{2=}$	39	28 –53
$C_{18}^{3=}$	7.9	5.1–13

Table 3 presents the mean distribution of total fatty acids in the sera of 12 Holstein cows sampled in a corollary study. The data indicate a general lack of short-chain fatty acids in the total lipids of bovine serum. This essentially eliminates the possibility that increased proportions of short-chain fatty acids (C_6-C_{14}) compensate for lack of long-chain unsaturated fatty acids ($C_{18}^{=} - C_{18}^{3=}$) in the serum of some normal Holstein cows. Thus, the shortage of short-chain fatty acids in the sera of the four cows used in the main study seems characteristic of the Holstein.

A comparison of fatty acid distribution between the whole serum total lipids in Tables 2 and 3 further confirms the representativeness of the original four cow-sample.

The high- and low-density lipoproteins in bovine serum show significant differences in their lipid moieties. Variations exist not only in weight proportions but in degree of saturation and fatty acid patterns. Few but notable similarities also are present. Further studies seem indicated regarding lipoprotein turnover, density subgroup distribution, preferential attack by lipases, relation to triglyceride dynamics, and a more complete characterization of the associated proteins and phospholipids.

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NUTRITIVE VALUE OF HIGH-MOISTURE CORN WHEN FED WITH VARIOUS SILAGES TO LACTATING DAIRY COWS

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SUMMARY

A feeding trial was conducted comparing the nutritive value of high-moisture corn when fed in combination with corn, oat, or sorghum silage to cows of varying productive capacity. An attempt was made to feed the high-moisture corn on an equal dry matter basis and any variation in the total dry matter consumption that did occur was removed by regression techniques before a final analysis was made. The efficiency of utilization of the dry matter of the high-moisture corn increased as the moisture content of the corn increased from 22 to 32% moisture. The dry matter of oat silage was used less efficiently for the production of milk than was the dry matter of corn or sorghum silage. The latter two were about equally well utilized for milk production.

The significance of body weight changes of cows fed the various high-moisture corns and silages was obscured by a second-order interaction. However, the lower-moisture corns fed in combination with oat silage to high-producing cows were less efficient in maintaining body weight than were other possible combinations.

The high-moisture corn fed in this trial was found to contain yeast rather than mold when removed from storage. No difficulties were observed when high-moisture corn was fed to lactating dairy cows.

The use of picker-shellers and other heavy corn-harvesting equipment has aroused an interest in early harvest of the corn crop to minimize the inconvenience of wet fields due to fall rains.

Boyd *et al.* (6) and Isaacs *et al.* (9) have shown that high-moisture corn can be stored in silos with little or no spoilage. Isaacs successfully stored high-moisture corn containing 25 and 30% moisture, and Boyd successfully stored ground shelled corn containing as high as 40% moisture.

The fact that corn can be harvested early and stored with little or no spoilage raises a question as to the nutritive value of high-moisture corn. Boyd *et al.* (6) compared the nutritive value for lactating dairy cows of ground shelled corn containing either 26 or 40% moisture and ground ear corn containing 36% moisture with a control corn containing 15% moisture. They noted significant differences during a 112-day experimental period in the per cent decline of 4% fat-corrected milk production between cows fed the various corn rations. The control and the 26% moisture corn groups declined 29 and 24%, respectively, whereas the 40% moisture ground shelled corn group and the 36% moisture ground ear corn group declined 46 and 40%, respectively. Boyd explained the large decline in milk production by cows fed the 36% moisture ground corn as an influence of the cob portion of the ration. However, ground ear corn is

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usually considered equal in feeding value to shelled corn for dairy cattle unless the concentrate portion of the ration is already high in bulky feeds (10).

It was the purpose of this project to further investigate the use of high-moisture corn as a feed for lactating dairy cows. The experiment was designed to evaluate the effect of moisture content of the corn when fed in combination with different silages to cows of varying productive capacity.

EXPERIMENTAL PROCEDURE

Nine Brown Swiss and 18 Holstein cows of the University of Illinois pure-bred herds were grouped according to milk production, kind of silage fed, and moisture level of the corn grain in a combination split-plot and switch-back design. The experimental plan is shown (Table 1). The 27 cows were assigned

TABLE 1

Assignment of cows

I. High producers

Roughages:	Corn silage and hay			Oat silage and hay			Sorghum silage and hay		
Cow No.	1392[a]	1419	1575	1469[a]	1651	1628	1567[a]	1516	1642
Periods (7 wk.)									
I	A	B	C	B	C	A	C	A	B
II	B	C	A	C	A	B	A	B	C
III	C	A	B	A	B	C	B	C	A

II. Medium producers

Roughages:	Corn silage and hay			Oat silage and hay			Sorghum silage and hay		
Cow No.	1481[a]	1515	1574	1249[a]	1458	1580	1452[a]	1570	1630
Periods (7 wk.)									
I	C	A	B	A	B	C	B	C	A
II	B	C	A	C	A	B	A	B	C
III	A	B	C	B	C	A	C	A	B

III. Low producers

Roughages:	Corn silage and hay			Oat silage and hay			Sorghum silage and hay		
Cow No.	1624[a]	1646	1532	1632[a]	1644	1647	1611[a]	1633	1648
Periods (7 wk.)									
I	B	C	A	C	A	B	A	B	C
II	C	A	B	A	B	C	B	C	A
III	A	B	C	B	C	A	C	A	B

[a] Brown Swiss.

Grain rations: A = 22% corn + S.O.M. mineral mixture; B = 26% corn + S.O.M. mineral mixture; C = 32% corn + S.O.M. mineral mixture.

to three major groups according to initial level of production. The daily 4% fat-corrected milk (FCM) production for the groups ranged from 51.8 to 67.0 lb. for the high-production group, from 36.9 to 51.9 lb. for the medium-production group, and from 30.0 to 38.4 lb. for the low-production group. The nine cows of each group were further randomly divided into subgroups, placing one Brown Swiss and two Holstein cows on a diet of corn, oat, or sorghum silage

for the entire course of the experiment. The three cows of each subgroup within a major group were alternated in a random fashion to provide an equal number in each sub-subgroup at all times. The sub-subgroups consisted of cows receiving rations of 22, 26, or 32% moisture shelled corn. The experiment was conducted for 21 wk., from November 1 to April 10, and was divided into three periods of 7 wk. each. The data obtained during the last 5 wk. of each period were used for analysis.

Each cow received daily 6 lb. of good-quality alfalfa hay, 1 lb. of grain per 3 lb. of FCM, and silage to a maximum of 1.5 lb. hay equivalent per 100 lb. live weight. The grain portion of the ration included high-moisture shelled corn fed at a rate to provide dry matter equal to that of corn containing 13% moisture and 2.0 lb. of soybean-oil-meal mineral mixture. When the corn allowance dropped below 6 lb. per day, the soybean-oil-meal mineral mixture was reduced to 1.0 lb. per day. Under no circumstance was the corn allowance reduced below 4 lb. per day.

The high-moisture corn had a fermented odor and a darkened appearance when removed from the silos in which it was stored. Samples taken at various levels within a foot of the top of each silo revealed yeast to be present rather than mold. However, the corn molded readily when it was removed from storage and allowed to stand at room temperature.

The cows were stabled in their normal stalls with no attempt at grouping. Wheat straw was used for bedding. Daily feed and milk records were kept. Milk samples were taken the first three days of each week and the fat content was determined by the Babcock method on composite samples. Feed samples were also taken the first three days of each week and the dry matter determined by drying at 62° C., in accordance with the findings of Colovas (7).

RESULTS AND DISCUSSION

A statistical analysis of the data for dry matter intake by cow groups is presented in Table 2. This analysis reveals no significant differences in the amount of dry matter consumed by the cows of varying productive capacity. The similarity in dry matter consumption is not unexpected, since grain was the most variable component of the ration. The grain portion of the ration was fed in proportion to the quantity of milk produced, and milk production varied an average of only 4 lb. per cow per day between the high- and low-producing groups.

When cows were grouped according to the type of silage fed, the cows in the group fed oat silage consumed significantly less dry matter ($P < .05$) than did cows either in the group fed corn silage or in the group fed sorghum silage. This relationship was consistent among high-, medium-, and low-producing cows and when the silages were fed with rations containing corn with 22, 26, or 32% moisture. This may be attributed in part to the fact that the oat silage contained more moisture than the two other silages. In addition, a capacity or a palatability problem resulted, for not all of the cows consumed the entire amount of silage allotted them. The oat silage group, in particular, suffered this diffi-

TABLE 2

Analysis of variance of dry matter intake

Source*	d.f.	Sums of squares	Mean square	F
Total	80	2,077.45		
Production level	2	273.23	136.62	2.54
Silages	2	496.25	248.13	4.62*
Corn vs. oat and sorghum	1	152.62	152.62	2.84
Oat vs. sorghum	1	343.63	343.63	6.40*
Silages × production level	4	53.80	13.45	.25
Animals in silage error (a)	18	966.95	53.72	
Periods	2	145.08	72.54	28.45*
Corn	2	16.48	8.24	3.23
Linear	1	16.47	16.47	6.46*
Quadratic	1	.01	.01	.004
Corn × silage	4	8.10	2.03	.80
Corn × production level	4	3.94	.99	.39
Corn × silage × production level	8	26.80	3.35	1.31
Error (b)	34	86.82	2.55	

* Significant at the 5% probability level.
* The single degrees of freedom not shown were found to be not significant ($P < .05$).

culty. The corn silage group more nearly approached complete consumption of its allotted portion than did the sorghum silage group, although the moisture contents of the corn and sorghum silages were nearly equal. This would suggest that the corn silage was slightly more palatable than the sorghum silage. The amounts of dry matter consumed by cows fed either corn or sorghum silage were nearly identical.

A significant linear relationship ($P < .05$) was observed in the dry matter intake of cows fed rations containing corn of different moisture content. The groups fed corn containing 22, 26, or 32% moisture consumed 804.5, 820.1, and 834.3 lb. of dry matter per day, respectively. The relationship was not evident in Periods 1 and 2, even though the combined data for the entire experiment showed a significant trend. The discrepancies in Periods 1 and 2 apparently are related to variations in milk production. Because of a last-minute substitution, the 32% moisture corn grain group began the experiment at a lower level of production and consumed less dry matter than the two other groups during the first period of the experiment. As the trial progressed, and the cows were switched back and forth to permit each cow to receive each type of corn grain available, the situation was reversed.

The level of milk production did not influence the amount of dry matter consumed by the silage groups or the high-moisture corn groups. Neither did the kind of silage fed cause a variation in the amount of dry matter consumed by the various high-moisture corn groups. However, as has already been noted, the amount of dry matter consumed varied linearly with respect to the moisture content of the corn grain, and the dry matter consumption was depressed in the case of the oat silage group. For these reasons, it was necessary in analyzing the body weight gains and the milk production data to adjust the data, using regression techniques (11) to remove the variation due to differences in dry matter consumption.

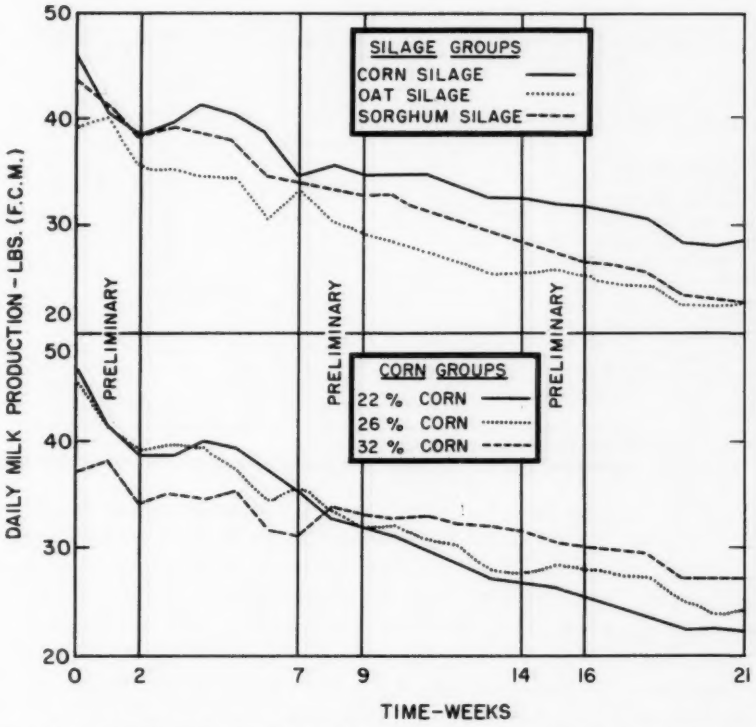

FIG. 1. Average milk production of cows in the corn silage and high-moisture corn groups.

Figure 1 presents the unadjusted daily milk production records of the silage and high-moisture corn groups. Although an attempt was made at the beginning of the trial to assign the cows to uniform groups, a discrepancy occurred in the 32% moisture corn group. This group had a lower initial level of production. However, the production curve of the 32% moisture corn group shows a greater persistency than do the curves for either of the two other high-moisture corn groups. The 32% moisture corn group declined an average of only 11 lb. of FCM per cow during the experimental period, whereas the 26% moisture corn group declined 15 lb., and the 22% high-moisture corn group declined 16 lb. of FCM during the experimental period.

The cows fed corn silage were slightly more persistent than those fed either sorghum or oat silage. During the experimental period, the daily milk production for the corn silage group declined an average of 10 lb. of FCM per cow, whereas the oat silage group declined an average of 13 lb., and the sorghum silage group declined an average of 16 lb. of FCM per cow.

A statistical analysis of the milk production data adjusted for variability in dry matter intake is presented in Table 3. The milk production of the high-

TABLE 3

Analysis of variance of milk production adjusted for dry matter intake

Source[a]	d.f.	Adjusted sums of squares	Adjusted mean square	F
Total	78	4,434.79		
Production level	2	568.94	320.76	2.63
Silages	2	1,108.82	554.41	4.56*
Corn vs. oat and sorghum	1	339.97	339.97	2.79
Oat vs. sorghum	1	768.85	768.85	6.32*
Silages × production level	4	103.82	25.96	.21
Animals in silage error (a)	17	2,068.74	121.69	
Periods	2	282.77	141.39	23.57*
Corn	2	27.81	13.91	2.32
Linear	1	27.52	27.52	4.59*
Quadratic	1	.29	.29	.05
Corn × silage	4	12.10	3.02	.50
Corn × production level	4	6.97	1.74	.29
Corn × silage × production level	8	56.93	7.11	1.19
Error (b)	33	197.89	6.00	

* Significant at the 5% probability level.

[a] The single degrees of freedom which are not shown were found to be not significant.

moisture corn groups varied directly with the moisture content of the corn. FCM production for the entire trial period was 801.1, 838.5, and 871.9 lb. per day, respectively, for the 22, 26, and 32% moisture corn groups. After removal of the variation due to differences in dry matter intake, this linear trend was shown to be significant ($P < .05$). The reason for the more efficient utilization of the dry matter in the 32% moisture corn is not readily apparent. A part of this effect might have been a result of the greater mechanical damage incurred in the harvesting and storing of the 32% moisture corn. However, the 26% moisture corn apparently received the least amount of mechanical damage and was intermediate in efficiency of utilization.

The oat silage group produced significantly less milk ($P < .05$) than did the two other silage groups. The daily milk production for corn, oat, and sorghum silage groups was 930.5, 750.3, and 837.7 lb., respectively. This relationship was consistent throughout all three periods, indicating that the dry matter of oat silage is used less efficiently for milk production than is the dry matter of corn or sorghum silages.

The FCM production of the high-, medium-, and low-producing cows was not significantly different after dry matter consumption variabilities were removed. The high-, medium-, and low-production level cows average 49, 44, and 37 lb. of FCM per cow per day, respectively, at the beginning of the experiment. There were no significant interactions in the feed efficiencies for the production of milk by cows fed the various silages and high-moisture shelled corns or by their level of milk production.

Table 4 contains the analysis of the body weight changes after adjustment for dry matter intake. The body weight changes were influenced by the type of silage fed, the moisture content of the corn grain fed, and the milk production of the cows. The interaction of these three factors produced significant body weight changes, although no significant effects were observed from any two of

TABLE 4

Analysis of variance of body weight gains adjusted for dry matter intake

Source[a]	d.f.	Adjusted sums of squares	Adjusted mean square	F
Total	78	101,162.37		
Production level	2	417.27	208.64	.39
I Linear	1	407.99	407.99	.77
II Quadratic	1	9.28	9.28	.02
Silages	2	11,824.19	5,912.10	11.10*
III Corn vs. oat and sorghum	1	6.69	6.69	.01
IV Oat vs. sorghum	1	11,817.51	11,817.51	22.19*
Silages × production level	4	1,233.98	308.50	.58
Animals in silage error (a)	17	9,053.39	532.55	
Periods	2	47,230.07	23,615.04	56.23*
Corn	2	5,071.51	2,535.76	6.04*
V Linear	1	4,852.43	4,852.43	11.55*
VI Quadratic	1	219.08	219.08	.52
Corn × silages	4	2,365.95	591.49	1.41
Corn × production level	4	3,633.04	908.26	2.16
Corn × silage × production level	8	6,473.81	809.23	1.93
V × IV × I	1	1,809.78	1,809.78	4.31*
VI × III × I	1	3,060.12	3,060.12	7.29*
Error (b)	33	13,859.16	419.97	

* Significant at the 5% probability level.

[a] The single degrees of freedom which are not shown were found to be not significant.

the factors. High-producing cows fed oat silage and 22% moisture corn grain lost more weight during the trial than did high-producing cows fed corn or sorghum silage and 26 or 32% moisture corn. The weight losses per cow per period after adjustment for dry matter consumed varied linearly with production level and linearly with moisture content of the corn. The high-, medium-, and low-producing cows lost an average of 38, 27, and 21 lb. of body weight per cow, respectively, per period. The 22 and 26% moisture corn grain groups lost an average of 54 and 36 lb., respectively, whereas the 32% moisture corn grain group gained an average of 3 lb. per cow per period. The oat and corn silage groups lost 74 and 28 lb. per cow per period, respectively, while the sorghum silage group gained an average of 15 lb. per cow per period. These differences might be due to variations in degree of fill, but when averaged over nine cows per group, there is an indication of a trend. For a clear-cut answer to the question of weight changes regarding a particular silage or moisture level of corn grain, the feed in question should be tested with no more than one of the other factors tested here.

The results obtained in this experiment regarding the nutritive value for milk production and weight gains of high-moisture corn correspond to the results obtained by Boyd *et al.* (6), who found a greater nutritive value for 26% moisture ground shelled corn than for lower-moisture corns. These authors also indicate that shelled corn containing 40% moisture and ground ear corn containing 36% moisture is inferior in nutritive value for lactating dairy cows to 26 or 15% moisture corn. The results of the present experiment further correspond to those obtained by Beeson *et al.* (1-5), and Culbertson *et al.* (8), who

report an increased feed efficiency of high-moisture corn of 31 and 32% moisture over 10 to 15% moisture corn for fattening beef cattle.

In general, the experimental evidence indicates that the nutritive value of high-moisture corn increases linearly as the moisture content of the corn increases up to approximately 32% moisture.

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NET ENERGY OF PINEAPPLE BRAN AND PINEAPPLE HAY WHEN FED TO LACTATING DAIRY COWS[1, 2]

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SUMMARY

By means of a switch-back design, the net energy values for pineapple bran and pineapple hay were determined as being 53.5 and 38.9 megacalories per 100 lb., respectively. Milk production and butterfat levels were maintained satisfactorily when pineapple hay and pineapple bran were used as a roughage source. Neither material had an adverse effect on milk flavor. Pineapple hay appears to be a potential source of medium-quality roughage for cattle feeding.

Hawaii's admittance as the 50th state in the Union has triggered a vigorous industrial and urban development of existing farm areas. The dairy industry has not escaped this encroachment. An earlier survey conducted by Philipp and Koshi (16) indicated that most of the dairy cows are fed and managed under dry-lot conditions and that approximately a third of the animals supplying the Honolulu milk market do not receive any form of farm-grown forages. Consequently, considerable tonnages of hauled-in soilage and imported roughages such as double-compressed alfalfa hay, wafers, pellets, and chopped hay are being used to supply the roughage needs of the cows.

Profitable dairy production is closely associated with an abundant source of economical, quality forage, but in areas where land is limited and high-priced, conventional forage production does not appear possible. Therefore, some other avenues of roughage supply must be sought. Studies with beef and dairy cattle (4-6, 13, 15, 17, 18) have shown that roughages of lower quality could be used with apparent good results when properly supplemented with other feedstuffs to formulate an adequate ration. The possibility of using dehydrated pineapple plant [*Ananas comosus* (L.) Merr.], hereafter referred to as pineapple hay, as a source of roughage for milking cows, was investigated to further elucidate its potential as a feedstuff in the ration of milking cows. Preliminary feeding trials (14) showed that chopped pineapple plants fed either as soilage or silage were comparable to fresh chopped napier grass (*Pennisetum purpureum*) in feeding value and milk production.

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[2] Approved by the Director of the Pineapple Research Institute of Hawaii as Technical Paper No. 273.

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The present study reports the net energy values of pineapple bran and pineapple hay when fed to milking cows and their effect on milk production and milk quality.

EXPERIMENTAL PROCEDURES

Twelve Holstein-Friesian cows past their lactation peaks, and whose records showed no more than mid-gestation at the close of the experiment, were selected and allotted to a combined switch-back, incomplete block design as developed by Lucas (10) with four treatments, three periods, and three blocks. Between cow variation in production and most of the variations due to differences in persistency were automatically removed from the experimental error by the use of this design. Only three of the treatments are of concern in this study. They consisted of:

1. 100% basal
2. 70% basal + 30% pineapple bran
3. 70% basal + 30% pineapple hay

The percentage composition of the basal ration consisted of pineapple bran, 40.0; soybean oil meal, 30.0; cane molasses, 15.0; rolled barley, 10.0; and coconut oil meal, 5.0. Trace-mineralized salt and dicalcium phosphate (50:50) were fed free-choice. A large amount of pineapple bran was used because pineapple bran is palatable, an economical feed produced locally, and a good source of roughage material. The experimental cows did not receive any form of additional roughage over and above that supplied in their respective rations in the form of pineapple bran or pineapple hay.

The protein intake of these cows was such that on Rations 1, 2, and 3 the protein furnished was sufficient for full milk production.

The feeding procedures, management of the experimental animals, collection of milk and feed samples, body weight measurements, fat tests, and gross energy determinations have been published earlier (8).

The total digestible nutrients (TDN), digestible crule protein (DCP), and digestible energy (DE) were determined on all rations in conventional digestion trials with sheep. Six trials each were conducted on the basal, basal + pineapple bran, and basal + pineapple hay. TDN was calculated from digestible organic matter (7). Gross energy of milk was calculated from a prediction equation, $Y = 35.09 + 8.89X$, where Y equals energy value of fluid milk in kilocalories (kcal.) per 100 g. and X equals per cent fat. This equation was developed from earlier work (8) involving the relationship of per cent fat to gross heat of combustion, involving 152 samples varying from 1.4 to 4.0% fat. The coefficient of determination (r^2) was 0.98 and the standard error of the regression coefficient ($b = 8.89$) was 1.79. Net energy (NE) values were determined from the basic concept, $NE = M + P$, where M equals energy for maintenance and P equals energy appearing in products such as milk and body weight gains. The methodology for the development of the equations was reported previously (8). Milk flavor and rancidity scores were determined by the quality control laboratory of the Honolulu Dairymen's Association, Honolulu, Hawaii.

Pineapple hay used in this experiment included the slips, suckers, immature fruits, stumps, and dead leaves as shown in Figure 1. The plants were hand-harvested, chopped, and dried in a triple-pass dehydrator. The pineapple plants are ready for harvest immediately after the last marketable ratoon fruits are picked. Based on a 3-yr. plant cycle and a statewide acreage of 77,000 acres, approximately 25,000 acres could be harvested for possible livestock feeding, with a potential dry matter yield of 15 to 20 tons per acre.

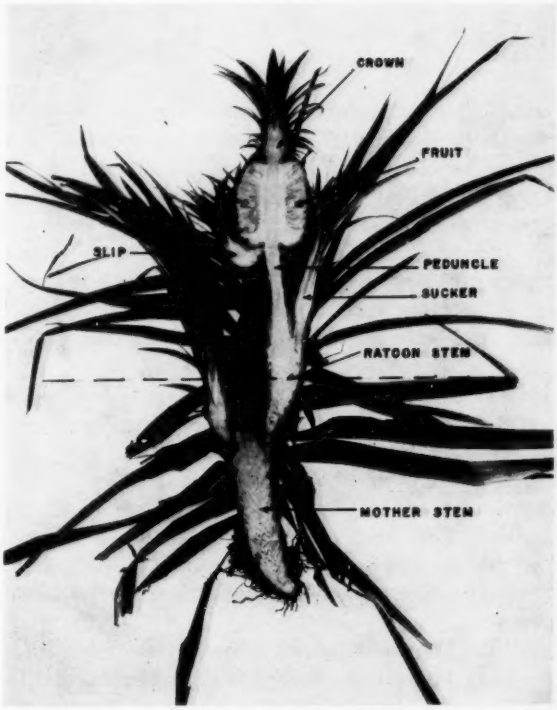

FIG. 1. Longitudinal section of pineapple plant showing various plant parts. Vegetative portions above dotted line to be used for possible commercial production.

RESULTS AND DISCUSSION

The results of the digestion trials are shown in Table 1. The digestibility trials demonstrated that pineapple bran was highly digestible and palatable. Pineapple bran, the dehydrated cannery residues consisting primarily of pineapple fruit shells and trimmings, is one of the by-products of the pineapple industry. It is not a bran by feedstuff definition but falls into the pulp category, such as beet and citrus pulp. A TDN value of 66.1 lb. per 100 lb. and a DE value of 118.0 megacalories (mcal.) per 100 lb. for pineapple bran compare favorably with dried spring and autumn pasture grasses and other forages on

TABLE 1
Composition and feeding value of pineapple bran and pineapple hay, as fed

Ration	Dry matter	Gross energy	DE	TDN	DCP
	(%)	*(kcal/g)*	*(kcal/g)*	*(lb/cwt)*	*(lb/cwt)*
Basal	87.10	3.37	3.07	71.18	16.61
Basal plus:					
30% pineapple bran	87.33	3.67	2.93	69.66	12.57
30% pineapple hay	87.93	3.77	2.77	65.75	13.00
By difference:					
Pineapple bran			2.60	66.13	0.00
Pineapple hay			2.07	53.08	1.47
Standard error of the mean, $s_{\bar{x}}$			± .05	± 0.64	

western mountain ranges (12). Pineapple bran is one of the major constituents fed to dairy animals in Hawaii and its demand far exceeds the normal supply of 20 to 25,000 tons per year. The advantage in unit cost of available energy per 100 lb. of pineapple bran in comparison with other carbonaceous feeds, coupled with a mounting demand for its use by other classes of livestock, has had an economic impact on the dairy industry.

The dehydrated pineapple hay was highly acceptable by the test animals. The corresponding TDN and DE values per 100 lb. of pineapple hay were 53.1 lb. and 93.5 meal., respectively. These values are in line with dry roughages such as clover hay or Hegari fodder.

The DCP content of pineapple bran and pineapple hay was negligible.

The results of this experiment are interpreted as setting the minimum values for TDN, DE, and NE for pineapple hay, since the entire plant (including senescent and dead plant parts which are low in feed value) was used. On a commercial basis, the top one-third of the ratoon pineapple plant would be used for feed production. This would eliminate dead plant material so that the starting material for pineapple hay would be comprised primarily of green, succulent plant material and this, in turn, should give rise to a better quality feed.

Table 2 shows that there were no statistically significant differences in milk energy or total energy production between the basal ration and the rations containing either 30% pineapple bran or pineapple hay. The total efficiency with which the DE was converted to energy in milk and energy in body weight changes showed the same trend, although there appeared to be some apparent differences. One of the problems associated with a change-over design is that the results of a given treatment may be affected by the previous treatments in the same sequence (11), but since in a previous study (8) there were no carry-over effects of somewhat similar rations, the evaluation of the response to treatment appears valid under the conditions of this study. The partial efficiency with which DE was converted to milk energy was somewhat lower than the average efficiency of 33% cited by Brody (1) for good cows. There were considerable differences between individual cows in their ability to convert DE to milk energy. Information of this sort may be useful in upgrading a milking herd for greater efficiency in milk production.

TABLE 2
Energy yield and efficiency of energy utilization

	Ration		
		Basal plus:	
	Basal	Pineapple bran	Pineapple hay
Body weight *(lb.)*	1,195	1,199	1,179
Daily feed intake, as fed *(kg.)*	11.00	10.86	11.53
Daily DE intake *(kcal.)*	29,088	28,342	27,847
Daily milk energy yield *(kcal.)*	7,691	7,215	7,762
Daily weight gain *(lb.)*	0.57	0.34	0.00
Daily energy equivalent to weight gain or loss *(kcal.)*[a]	1,112	663	000
Daily total energy yield *(P) (kcal.)*	8,803	7,878	7,762
Total energy efficiency *(%)*[b]	30.26	27.80	27.87
Partial energy efficiency *(%)*[c]	26.44	25.46	27.87

[a] One pound of body weight change is equal to 1.95 meal. (8).

[b] $\frac{100 \times \text{total energy yield}}{\text{DE intake}}$.

[c] $\frac{100 \times \text{milk energy yield}}{\text{DE intake}}$.

The calculation of the NE of the basal ration, pineapple bran, and pineapple hay is presented in Table 3.

The lower level of NE required for maintenance of dairy cows given by Morrison (12) is described by the equation, $NE = 0.0137\ W^{0.87}$, where NE is expressed as megacalories per day and W as pounds in body weight. By use of this equation, one can calculate the NE required for maintenance (M) for any given body weight. Since the total energy yield (P) includes the correction for body weight changes, the basic concept for the determination of NE, $NE = M + P$, is satisfied.

TABLE 3
Calculations of NE of pineapple bran and pineapple hay for milk production

		Basal + 30%	
	Basal	Pineapple bran	Pineapple hay
Body weight *(lb.) (W)*	1,195	1,199	1,179
NE for maintenance *(mcal.) (M)*[a]	6.52	6.54	6.44
Total energy yield *(mcal.) (P)*[b]	8.80	7.88	7.76
Total NE in ration *(mcal.) (M + P)*	15.32	14.42	14.20
Feed intake *(kg/day) (F)*	11.00	10.86	11.53
NE of Ration			
Kilocalories per kilogram[c] (NE)	1,393	1,328	1,232
Megacalories per 100 lb.	63.2	60.3	55.9
NE of additive			
Kilocalories per kilogram[d]	....	1,177	857
Megacalories per 100 lb.	....	53.5	38.9

[a] $M = 0.0137\ W^{0.87}$.

[b] From Table 2.

[c] $\frac{(M+P)}{F} \times 1{,}000$.

[d] $\frac{\text{NE of basal + additive} - 0.7 \times \text{NE of basal}}{0.3}$.

By comparing the groups fed the basal plus the two additives, the NE of pineapple bran and pineapple hay was found to be 53.5 and 38.9 mcal. per 100 lb., respectively, as shown in Table 3. Estimated net energy (ENE) values of 61.0 and 54.2 mcal. per 100 lb. for pineapple bran for dairy cows and other animals, respectively, as reported by Morrison (12), are based on the premise that the same feedstuff has a higher NE value for milk production than for fattening. The NE of 53.5 mcal. per 100 lb. of pineapple bran obtained in this study is the result of an additive effect of 30% pineapple bran to the basal ration which already contained 40% pineapple bran.

There was a close agreement of 63.2 and 65.1 mcal. per 100 lb., respectively, between the actual and calculated NE values of the basal ration. The latter was based on Morrison's (12) ENE values for various feed ingredients. By way of comparison, the NE of pineapple hay fed to beef steers (9) was 30.3 mcal. per 100 lb., as determined by the slaughter technique method. The corresponding TDN and DE values were 47.5 lb. per 100 lb. and 101 mcal. per 100 lb., respectively. The apparent variability of the NE values of 38.9 and 30.3 mcal. per 100 lb., obtained for pineapple hay from the two classes of livestock, can be explained by the fact that NE values for body maintenance or milk production are usually somewhat higher than for fattening. It has been reported (3) that the same feeds may have decidedly different NE values for various productive purposes, plane of nutrition, and other factors.

Data on milk production and composition are presented in Table 4. The butterfat test was low on all treatments but tended to be higher on the pineapple hay ration. The crude fiber content of pineapple bran and pineapple hay, which constituted the greater proportion of the ration, was 20.0 and 23.4%, respectively. Consumption of fiber per se amounted to a low of 2.35 lb. to a high of 3.74 lb. per cow per day between treatments. Molasses was incorporated into the basal and basal plus additive rations at 15.0 and 10.5%, respectively. An earlier study (8) showed that the addition of 10% molasses to a simple ration had no significant effect on fat test. The pineapple hay–fed cows appeared to produce milk of better acceptability, as indicated by the slightly lower average

TABLE 4

Response of cows to added increment of pineapple bran and pineapple hay

		Basal plus	
	Basal	Pineapple bran	Pineapple hay
Daily 4% FCM production *(lb.)*	23.13[a]	21.54[a]	23.56[a]
Butterfat *(%)*	2.54[b]	2.67[b]	2.98[a]
Rancidity [c]	0.92[a]	0.83[a]	0.65[a]
Flavor score [d]	35[a]	35[a]	35+[a]

[a, b] Values in the same category with common subscripts are not significantly different by Duncan's multiple range test(2).

[c] Milligrams KOH to neutralize free fatty acid in 1 g. of butterfat.

[d] The treatment mean flavor scores fell within the following ranges and descriptions:

Score	Description
36	Slight feed flavor
35–34	Distinct feed flavor

rancidity score and the slightly higher average flavor score. However, these differences were not statistically significant.

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EFFECT OF A COMPLEX MINERAL-VITAMIN SUPPLEMENT ON MILK PRODUCTION

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SUMMARY

Four experiments were conducted with high-producing Holstein cows to measure the influence of a complex mineral-vitamin supplement on milk production. In the different experiments, widely varying types and qualities of forages were fed. Different hay to grain ratios were tested and various ingredients were used in the grain mixtures. The mineral-vitamin mixture had no measurable influence on milk or fat production, body weight gain, general appearance, health, or on the efficiency of milk production.

Dairy farmers frequently inquire concerning the value they might obtain from feeding complex mineral-vitamin mixtures currently available on the market. Animals are known to need some 14 mineral elements. These mineral elements are also required for plant growth. Consequently, the feed of animals is never entirely lacking in any of these minerals and they are usually present in adequate amounts in good-quality feeds. Research has shown that rations of milking cows must be supplemented with common salt. Calcium, phosphorus, and iodine are often deficient in ordinary feeds and in some areas cobalt and copper need to be added to the rations of cattle.

Dairy cattle also require vitamins A, D, and E in their rations, but research has failed to demonstrate a dietary need for vitamin K or any of the B vitamins because of bacterial synthesis in the rumen.

The National Research Council (3) has summarized the mineral and vitamin requirements of dairy cattle. Many commercially mixed dairy rations contain supplements of certain of the mineral elements which exceed the amounts listed by the N.R.C. report. Some of them also contain supplements of vitamins A and D, even though most of the animals consuming the mixed feeds obtain ample amounts from the forages they consume. For commercial feeds distributed widely there is some justification for supplementation at a level which will meet the requirements of cattle fed the poorest quality forage, since the insurance cost is small.

In vitro rumen fermentation studies and certain fattening trials with beef cattle fed corn cobs or cotton seed hulls seem to have influenced many people to believe that complex mineral and vitamin supplements are necessary for optimum rumen function, regardless of the quality of the forage fed. These complete supplements are expensive, adding several dollars to the cost of a ton of mixed dairy feed, thus representing a tremendous waste if, in fact, they are not needed in most instances, a view supported by the NRC report (3).

Because of the lack of recent research on the question, except for the one comparison made by Moody *et al.* (2), a series of tests was conducted to study

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the effects on milk production of adding to a supposedly adequate ration a complex mixture containing the essential mineral elements and vitamins.

EXPERIMENTAL PROCEDURE

The mineral-vitamin supplement studied is shown in Table 1. In four feeding experiments, half of the cows in each experiment were fed a total of 70 g. of the mineral-vitamin mixture daily in two feedings and the other half received no supplement. The 70 g. fed daily would be approximately the amount a cow would receive in 15 lb. of grain if the vitamin-mineral mixture was added to a dairy feed at the rate of 20 lb. per ton. The supplement feeding was started at 8 to 10 wk. after calving and as the cows reached their peak of production.

TABLE 1

Composition of the mineral-vitamin supplement

Ingredient	Pounds
Soybean oil meal (50% protein)	800
Mineral-vitamin premix [a]	500
Dicalcium phosphate	440
Cane molasses	100
Ground limestone	80
Corn gluten meal	60
Wheat germ meal	20
Total	2,000

[a] The 500 lb. of mineral-vitamin premix contained 101.64 lb. of soybean oil meal (50% protein), 100 lb. of linseed oil meal, 100 lb. of irradiated 9-F yeast (9,000 I.U. vitamin D per gram), 60 lb. of dextrose, 60 lb. of manganese sulfate, 20 lb. of wheat germ oil, 20 lb. of lecithin, 12.33 lb. of choline chloride (25%), 10 lb. of iron oxide (black), 3.86 lb. of calcium gluconate, 2.2 lb. of ascorbic acid, 2.0 lb. of BY-21 (8,000 μg. riboflavin per gram), 1.6 lb. of vitamin A supplement (325,000 units vitamin A per gram), 1.5 lb. of potassium iodide, 1.5 lb. of copper gluconate, 0.46 lb. of cobalt carbonate, 0.4 lb. of zinc carbonate, 0.05 lb. of calcium pantothenate, and 0.01 lb. of thiamine hydrochloride.

The experiments in which the vitamin-mineral mixture was tested also included other dietary comparisons. A brief outline of each test is given here, but full details of the related tests will be published elsewhere.

Experiment I. Two different 16% protein grain mixtures were tested with half of the cows on each, selected as pairs, receiving the mineral-vitamin supplement. The same allotment plan was followed in the other experiments. Average quality timothy hay and corn silage were fed according to appetite and 1.0 lb. of grain was allowed for each 3.5 lb. of FCM.

Experiment II. Twelve Holstein cows received a uniform type of alfalfa hay and a grain mixture at different grain to hay ratios. Hay furnished 60, 40, or 20% of the net energy in the different rations and the concentrates supplied the balance. Details of the study have been published (1).

Experiment III. Twenty-two Holstein and two Brown Swiss cows were used to compare the response to early- and late-cut forages. Each cow received the assigned forage to its maximum consumption for the duration of the experiment. In addition, one-half of the cows received grain at a 1:4 ratio and the other at a 1:8 ratio (4). Breed of cows was not considered in the allotment to treatment.

TABLE 2

Responses of the cows

Item	Experiment No. and treatment							
	I		II		III		IV	
	Control	Supplement[a]	Control	Supplement[a]	Control	Supplement[a]	Control	Supplement[a]
No. of cows	8	8	6	6	11	11	8	8
Length of trial *(wk.)*	18	18	18	18	20	20	16	16
Av. FCM yield *(lb.)*	5,479	5,451	4,990	4,919	5,820	5,614	5,399	5,311
Av. daily FCM yield *(lb.)*	43.5	43.3	39.6	39.0	41.6	40.1	48.2	47.4
Av. TDN intake *(lb.)*	2,689	2,794	2,940	2,797	2,862	2,738	2,431	2,637
Av. daily TDN intake *(lb.)*	21.3	22.2	23.3	22.2	20.4	19.6	21.7	23.5
Av. body weight *(lb.)*	1,125	1,159	1,356	1,218	1,340	1,239	1,354	1,339
Av. weight gain *(lb.)*	67	74	22	−72	27	50	−0.9	11
Av. daily weight gain *(lb.)*	.53	.59	.17	−.57	.19	.36	−.008	.098
FCM yield/lb TDN[b]								
Gross	2.04	1.95	1.70	1.76	2.03	2.05	2.22	2.01
Net	4.08	3.90	3.19	2.72	4.35	4.56	4.00	3.40

[a] The vitamin-mineral supplement formula contained in Table 1.

[b] The gross value is based on the FCM yield and the total TDN consumed. The net value is corrected for the TDN used for maintenance (NRC requirements) and weight gain, assuming that 3.53 lb. of TDN were required for each pound of gain, and 2.73 lb. of TDN for each pound of weight lost.

Experiment IV. Sixteen Holstein cows were used to determine whether ear-corn silage could replace part of the 16% protein grain mixture. All cows received the same hay (legume, grass mixed) to their maximum consumption. Soybean oil meal was added to ear-corn silage to bring the protein intake comparable to the cows fed the regular 16% protein grain mixture. The grain was fed at a 1:3.5 ratio of FCM.

The milk produced was weighed at each milking and fat tests made on composite samples of four milkings each week. All of the feed allowed and refused was weighed and composite samples of the feeds were analyzed. The cows were weighed on three successive days at the beginning and end of each test period to measure weight changes.

RESULTS

The results of the study are presented in Table 2. None of the small differences seen in production, feed intakes, or weight changes was statistically significant. There were no differences in the health or general appearance of the cows as related to the supplement.

In Experiment III one of the cows was lost from the study after 16 wk., from a cause not related to the dietary treatments and its pair-mate was also excluded from the final calculations. Therefore, the data in Table 2 show only 11 pairs of cows instead of the 12 started. A consideration of the data for all of the cows for the first 16 wk. of the test revealed no difference in production trends, whether or not the extra pair of cows was included. It seemed more desirable to use the 20-wk. data for 11 pairs of cows rather than 12 pairs for 16 wk. It was also noted that the two Brown Swiss cows declined in production at almost exactly the same rate as Holstein cows on the same treatment as anticipated, and that ignoring breeds in the allotment, because of insufficient

TABLE 3
Digestion coefficients of the rations fed in Experiment II

Grain in ration	Cow No.	Digestion coefficients						
		Dry matter	Protein	Fat	NFE	Fiber	TDN	Energy
(%)		*(%)*						
					Controls			
60	194	65.7	74.9	59.6	76.0	50.4	64.5	65.5
	164	63.8	75.0	52.6	74.4	47.0	61.8	64.3
40	266	67.4	73.9	73.8	78.6	46.5	67.5	67.3
	174	66.8	75.4	71.0	77.8	46.2	66.8	67.7
20	251	71.7	76.1	78.2	80.8	48.4	72.9	72.3
	180	72.5	75.0	80.8	80.2	45.7	74.2	72.2
	Mean	68.0	75.0	69.3	78.0	47.4	68.0	68.2
					Supplement			
60	314	62.6	74.9	54.2	73.7	44.1	60.8	62.8
	254	67.8	75.8	67.2	77.8	44.8	67.2	67.8
40	318	69.0	76.2	70.6	77.1	52.2	69.3	68.8
	279	69.6	76.3	74.3	77.8	46.8	70.1	69.2
20	303	72.0	77.7	76.0	79.5	49.7	73.0	72.4
	321	71.5	74.5	78.8	79.4	50.7	72.9	71.7
	Mean	68.8	75.9	70.2	77.6	48.1	68.9	68.8

numbers of cows to select from, apparently did not bias the results. Fortunately, the other experiments did not encounter problems of this type.

Simultaneously with the feeding trial, a digestion trial was conducted with the cows in Experiment II. The digestion coefficients are presented in Table 3.

The differences in the mean digestion coefficients were very slightly in favor of the supplement in all cases except for NFE, but not significantly so.

TABLE 4

Average FCM, TDN intake, gain and efficiency of production

	Control	Supplement	Difference
Av. daily FCM *(lb.)*	43.2	42.4	0.8
Av. daily TDN intake *(lb.)*	21.7	21.9	0.2
Av. daily gain *(lb.)*	0.22	0.12	0.10
FCM per lb. gross TDN *(lb.)*	2.00	1.94	0.06

Table 4 presents the average data for all four experiments. It is entirely clear that the mineral-vitamin supplement exhibited no measurable favorable influence on the milk or fat production of the cows, or on the efficiency of milk production. Changes in body weight, general appearance, and health were no different on the supplement than on the control ration. Likewise, the mineral-vitamin supplement did not increase appetite for hay or silage and it had no influence on the digestibility of the rations tested. Since the rations fed differed widely in forage type and quality, in ratio of roughage to concentrates, in type of silage, and in the ingredients used in the different grain mixtures, the authors believe the negative results probably can be applied to many of the different conditions which occur on dairy farms.

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EFFECT OF STAGE OF MATURITY AT FIRST CUTTING ON QUALITY OF FORAGES [1,2]

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SUMMARY

A field containing approximately equal proportions of alfalfa, clover, and timothy, and a second field containing orchard grass, were cut for hay on three dates: May 25, June 9, and June 24, 1959. The hays were evaluated by digestion trials with sheep, and all except the latest-cut orchard grass by a lactation trial with Holstein cows. A 15-day delay in cutting date caused a significant reduction in milk production, less body weight gain, lower hay intake, and lower digestibility. When cut on the same date, feeding of the mixed hay resulted in more milk production, greater body weight gains, more intake, and higher digestibility than the orchard grass. When compared at similar stages of maturity, there was no significant difference in milk production or digestibility. However, even at similar stages of maturity, there was a species difference in intake. About 65% of the variations in calorie intake associated with stage of maturity could be attributed to differences in dry matter intake.

In recent years, increased emphasis has been placed on high-quality forages, and the effect of stage of maturity or date of harvest on quality. Reid *et al.* (10) have found that the percentage of digestible dry matter (DDM) of first-cut forages in New York could be accurately predicted by the equation $Y = 85.0 - 0.48X$, where Y = percentage DDM and X = days after April 30. Their studies indicated that species of plant had relatively little or no effect on the energy value of the forage. They also noted a linear decline in maximum intake from 2.5–3.0 lb. of hay-equivalent per 100 lb. of body weight for forage harvested in early June to 1.1–1.7 lb. for forage harvested in mid-July when dairy cows were used as test animals and concentrates were fed at the rate of 1 lb. for each 3 or 4 lb. of milk produced. A number of recently reported experiments with lactating cows have demonstrated the superior quality of early-cut forages for dairy cows (5, 10, 11, 15). However, no milk production data were found which compared relatively pure stands of one species of first-cutting forage with a second species when both were cut on the same date or at comparable stages of maturity. It is conceivable that differences in over-all feeding value between species cut on the same date may exist as a result of variations in intake by cows, and for a significant spread in digestibility to exist between the species due to dissimilarities in stage of maturity.

The objectives of this study were to determine the extent of the effect of stage of maturity at first harvest on the quality of a mixture of alfalfa-timothy-

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[2] The data in this paper are in part from a thesis presented by the senior author to the Graduate School of The Pennsylvania State University, in partial fulfillment of the requirements for a M.S. degree.

clover, and on that of a relatively pure stand of orchard grass *(Dactalylis glomerata)*. It also was desired to determine whether differences existed between the mixed forage and the orchard grass, since orchard grass is more mature than alfalfa, timothy, or clover when they are cut on the same date. In this study, quality was defined in terms of chemical analyses, digestibility by sheep, and rate of intake and lactation response by cows fed the forages.

EXPERIMENTAL PROCEDURE

One field containing approximately equal proportions of alfalfa, timothy, and clover, and a second field containing orchard grass, were divided into strips and cut for hay on three dates: May 25, June 9, and June 24, 1959. Alfalfa, timothy, and clover were all at a similar stage of maturity, while orchard grass was distinctly more mature on a given date, Table 1. The clover in the field of

TABLE 1

Stage of maturity of forages at dates of harvest

Hay No.	Date cut (1959)	Stage of maturity			
		Alfalfa	Clover	Timothy	Orchard grass
1	May 25	Early bud	Bud	Vegetative	.....
2	June 9	1/10 Bloom	Full bloom	Just past boot stage	.....
3	June 24	3/4 Bloom	Past full bloom	Full head	.....
4	May 25	.....	.....	.....	Flowering
5	June 9	.....	.....	.....	Headed and at mature pollen
6	June 24	.....	.....	.....	Seed at milk stage

mixed forage was predominantly red clover, but contained a noticeable amount of Ladino and alsike clovers. Manure had been applied to both fields during the previous winter. The field of mixed forage had been fertilized at a recommended level, but no commercial fertilizer had been applied to the orchard grass that season. All hays were partially field-cured and finished with heat. None was damaged by rain.

Hays 1–5 were used in a 54-day feeding trial with lactating cows to determine relative feeding values. Because of acreage limitations, there was not a sufficient amount of Hay 6 harvested to feed during the lactation trial. Fifteen Holstein cows were divided into five groups of three each on the basis of production, age, and body weight. One first-lactation heifer and two older cows were included in each group. All animals were in at least the 6th wk. of their current lactation at the beginning of the trial, and in not more than the 5th mo. of gestation at the end of the trial. The cow groups were designated A, B, C,

D, and E. Each group of cows was fed one type of hay for 27 days, and then switched to a second type of hay for another 27 days in a randomized block design of the type shown in Table 2.

The cows were established on a standardized diet of hay and grain prior to the beginning of the trial, as suggested by Lucas (7). This standardization period consisted of 12 days in which the cows were fed alfalfa of apparently excellent quality ad libitum. During the final nine days of the standardization

TABLE 2

Experimental design of lactation trial

Period	Cow group				
	A	B	C	D	E
I (Oct. 15–Nov. 10)	Hay 1	Hay 2	Hay 3	Hay 4	Hay 5
II (Nov. 10–Dec. 7)	Hay 3	Hay 5	Hay 4	Hay 2	Hay 1

period the cows were fed the concentrate mixture which was to be used during the trial, and each cow was rationed concentrate on the basis of Morrison's Table VIII A (9) for good roughage fed at the rate of 2 lb. per hundredweight. The amount of concentrate each cow received during the experimental periods was determined as follows: At the beginning of the trial its 4% FCM production during the prior period of October 7–13 was applied to Morrison's Table VIII A (9) for cows on very liberal feeding of good roughage, in order to arrive at a recommended allowance. The recommended allowances then were reduced by 20% for each cow, and the resultant amounts were fed at the beginning of the trial. Concentrate allowances subsequently were reduced 10% of the quantity being fed at the date of reduction for each cow at 2-wk. intervals under an equalized feeding plan (6). A 20% crude protein concentrate mixture was fed to all cows to prevent any possible protein deficiencies on the grass hays.

Each cow was fed the experimental hay as the only source of roughage, and to the point of maximum consumption. The amount offered was adjusted according to appetite, so that there was 1–3 lb. refusal daily. The daily ration of hay for each cow was weighed into canvas bags and fed in two portions. The refusals were weighed once daily and composited in barrels for sampling. Five bales which were soon to be fed were sampled weekly from each type of hay with a Penn State Forage Sampler. The same device was used to obtain samples from the hay refusals as often as the barrels containing the refusals became filled. All samples were composited and stored until the trial was completed. Proximate analysis according to A.O.A.C. procedures (1) was conducted on the composited samples of the hays and the composited samples of the refusals.

The cows were milked twice daily and the milk weights recorded. Once each week milk samples were composited from two consecutive milkings and tested for butterfat by the Gerber method (8). A period of six days at the beginning of each period was allowed for adjustment to the rations. The comparisons for milk production and hay consumption were based on the last 21 days of the experimental period.

The cows were weighed on the two days preceding the start of the trial, the last day of Period I, the first day of Period II, and the last two days of the trial. Weights were taken at the same time in the morning after the cows had been milked.

The per cent digestible energy, digestible dry matter, and digestible protein of all six forages were determined by feeding them to six groups of two sheep each. The forages were fed at the rate of 800 g. per sheep daily, with a seven-day preliminary period and a ten-day collection period. Quantitative collections of feces were made by using metabolism crates of a type previously described (3). Energy determinations were made on the dried, ground feces and on the forages by using an oxygen bomb calorimeter.

RESULTS AND DISCUSSION

One animal in Group E was removed from the lactation trial at the end of Period I due to severe digestive upsets. Data concerning it were omitted from all calculations and analyses.

The separate effects of periods, cow groups, and hays on milk production during the 21-day comparison period were determined by fitting of constants (12). The adjusted average daily production of 4% FCM was 38.8 lb. in Period I and 31.6 lb. in Period II. As expected, some difference did exist in production levels among cow groups. The adjusted daily production of 4% FCM per cow by Groups A through E averaged 33.4, 33.0, 37.7, 35.2, and 37.2 lb., respectively.

When the effects of cow groups and experimental periods were removed, daily FCM production per cow was 40.4, 37.1, 32.8, 34.3, and 31.4 lb. for Hays 1 through 5, respectively, Table 3. Since all the intervals between cutting dates were 15 days, the effect of a 15-day delay in cutting date was found by comparing average production by the cows fed Hays 1 and 2, Hays 2 and 3, and Hays 4 and 5. The lowered production of 3.5 lb. daily per cow, due to a

TABLE 3

Effect of type of hay on animal response by lactating cows

Hay fed [a]	Av. daily FCM per cow [b]	Av. daily changes in body weight	Av. daily hay intake per 100 lb. of body weight [c]	Av. daily intake of concentrates per cow
	(lb.)			
1	40.4	+1.4	2.92 ± 0.24 [d]	9.3
2	37.1	+0.9	2.63 ± 0.34	9.5
3	32.8	+0.6	2.24 ± 0.23	8.9
4	34.3	0.0	2.29 ± 0.31	9.0
5	31.4	−0.5	1.69 ± 0.34	9.5

[a] Hay 1—alfalfa-timothy-clover harvested 5-25; Hay 2—alfalfa-timothy-clover harvested 6-9; Hay 3—alfalfa-timothy-clover harvested 6-24; Hay 4—orchard grass harvestel 5-25; Hay 5—orchard grass harvested 6-9.

[b] Adjusted for differences in production levels among groups.

[c] Intake on 90% DM basis.

[d] Standard deviation.

15-day delay in cutting date, was significant ($P<0.01$). Hays 1 and 2 cut on the same respective dates as Hays 4 and 5 allowed a comparison of the mixed hay and the orchard grass cut on the same date. The difference of 5.9 lb. daily per cow in favor of the mixed hay was significant ($P<0.01$). Since the orchard grass on a given date was at a stage of maturity similar to that of the mixed forage harvested 15 days later, Table 1, a comparison could also be made of the production by cows fed the two types of forage harvested at similar stages of maturity by comparing the production of cows fed Hays 2 and 3 with those fed Hays 4 and 5. The difference of 2.1 lb. daily per cow in favor of the mixed hay as compared to the orchard grass cut at similar stages of maturity was not significant ($P>0.05$). It should be pointed out that a longer feeding period or higher production levels could be expected to increase the magnitude of the differences in milk production due to effects of hay quality. Lower genetic production potential of the animals, a larger percentage of concentrates in the ration, and/or the addition of other roughage in the ration would be expected to lessen the effects.

The average changes in body weight were +1.4, +0.9, +0.6, 0.0, and −0.5 lb. per cow daily for Hays 1 through 5, respectively, Table 3. It is recognized that these values are subject to considerable error, since the weights used in their calculation are averages of only two consecutive days' weights pre-trial, mid-trial, and post-trial. No statistical comparison of the body weight changes was made for this reason. However, it is felt that the trends are of some importance, since the changes are in good agreement with the milk production responses and the results of other similar type trials (5, 10).

The effect of type of forage on hay intake, Table 3, presents a trend similar to those of milk production and body weight changes. There was a definite lowering of intake with advancing maturity, and intake was more closely related to stage of maturity than to date of cutting in the forages tested. When expressed in terms of hay intake for a 1,300-lb. cow, a delay of 15 days in cutting resulted in a reduction of 5.6 lb. of hay daily, which was significant ($P<0.01$). The effect of species on intake was even more pronounced, with a 10.5 lb. difference in favor of the mixed hay fed to a 1,300-lb. cow when both forages were cut on the same date. When considered at similar stages of maturity, there was still a 5.7-lb. difference in daily intake per cow in favor of the mixed hay. These data indicate that a study of the effect of stage of maturity on dry matter intake, with special reference to species differences, would be in order.

Although the daily concentrate consumption varied from 4 to 20 lb. for individual cows, the average daily intake per cow was similar for all groups (Table 3). It is believed that the possibility of protein deficiencies influencing the results of this trial was eliminated by the use of a 20% crude protein concentrate. All cows received at least 110% of Morrison's (9) higher protein recommendations for maintenance and production at all times during the trial.

Results of digestibility trials using sheep fed the six hays are presented in Table 4. One sheep fed Hay 5 at times refused to eat, and the other one suffered

TABLE 4

Nutritive value of the hays determined by feeding to sheep (dry matter basis)

Hay fed [a]	Digestible protein	Digestible dry matter	Digestible energy
	(%)		
1	14.0	71.1	69.2
2	9.7	65.6	62.6
3	7.9	60.6	58.3
4	8.2	65.8	63.8
5	5.2[b]	50.4[b]	47.6[b]
6	4.8	55.8	53.4

[a] Hay 1—alfalfa-timothy-clover harvested 5-25; Hay 2—alfalfa-timothy-clover harvested 6-9; Hay 3—alfalfa-timothy-clover harvested 6-24; Hay 4—orchard grass harvested 5-25; Hay 5—orchard grass harvested 6-9; Hay 6—orchard grass harvested 6-24.

[b] One animal refused to eat Hay 5 and the other animal suffered a digestive upset during the course of the trial.

from digestive upsets. Application of the formulae used by the Pennsylvania Forage Testing Service (2) for estimation of per cent metabolizable energy from per cent crude protein and per cent crude fiber of the forage, and Swift's (14) equation for the relationship between metabolizable and digestible energy, gave an estimated value of 55.7% digestible energy for Hay 5. The reduction in digestible protein of the more mature forages agrees with other reports (5, 10). A delay of 15 days in the cutting date resulted in an average decrease in digestible energy of 6.3 percentage units. Using the estimated value for Hay 5, orchard grass averaged 5.7 percentage units lower in digestible energy than the mixed hay cut on the same date. When orchard grass was compared with mixed hay cut at a similar stage of maturity, the difference in per cent digestible energy of 0.7 percentage unit was negligible. The per cent digestible dry matter followed a trend very similar to that of the per cent digestible energy. These results are in contrast to the findings of Reid *et al.* (10), that species has little or no effect on the energy value of a forage cut on a given date. The fact that there was a difference in intake by lactating cows (Table 3), even when the forages were compared at similar stages of maturity and when the energy value was not different, illustrates that a difference in over-all feeding value may exist between forages in spite of energy being similar.

If the digestibility data for sheep can be applied to dairy cows, as the data of Swift and Bratzler (14) indicate, then the total differences in caloric intake among the forages may be partitioned into the differences in dry matter intake and differences in per cent digestible energy. It was calculated that about 65% of the reduction in caloric intake of the forages associated with advancing stage of maturity was due to reductions in dry matter intake, while only about 35% was due to changes in per cent digestible energy. These data are only slightly different from the findings of Crampton *et al.* (4) who, working with sheep, reported that relative intake appeared to determine about 70% of their Nutritive Index, while digestibility determined about 30%. The effect of stage of maturity of pure stands of various types of forage on maximum intake might give a different relationship than that which is reported in this study.

TABLE 5

Proximate analysis of hays fed and of the refusals in the lactation trial (dry matter basis)

Hay No.[a]	Ash	Crude protein	Ether extract	Crude fiber	N.F.E.
			(%)		
Hay fed					
1	7.6	18.5	2.3	25.1	46.5
2	5.5	13.3	2.2	30.2	48.8
3	5.2	12.2	1.9	32.8	47.9
4	7.9	12.4	2.8	31.4	45.5
5	5.6	9.6	2.4	35.2	47.2
Hay refused					
1	10.9	19.0	2.4	20.9	46.8
2	7.5	13.1	2.2	30.2	47.0
3	5.4	10.1	1.6	37.0	45.9
4	14.2	12.1	2.4	27.4	43.9
5	5.5	6.9	1.7	38.2	47.7

[a] Hay 1—alfalfa-timothy-clover harvested 5-25; Hay 2—alfalfa-timothy-clover harvested 6-9; Hay 3—alfalfa-timothy-clover harvested 6-24; Hay 4—orchard grass harvested 5-25; Hay 5—orchard grass harvested 6-9.

The results of proximate analysis of the hays fed and of the refusals in the lactation trial showed an interesting trend in selectivity of the forages, Table 5. In all cases the per cent crude fiber increased at a more rapid rate in the refusals than in the hay fed as cutting date progressed. The reverse trend was noted with respect to per cent crude protein. This indicates that the cows were more selective in choosing those parts of the plant high in protein and low in crude fiber in the more mature forages than in the early-cut forage.

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INFLUENCE OF WEATHER ON SUGAR CONTENT OF FORAGE CROPS[1]

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SUMMARY

Data from 72 lots of forage were submitted to statistical analysis to ascertain the relationships between their total sugar content and certain weather phenomena previous to harvest. Calculated values of r showed a significant inverse relationship between sugar content and average air temperature, slightly significant correlation between sugar content and rainfall, ($P = .05$), and little or no correlation with exposure to bright sunshine. The results are discussed in the light of the findings of other workers, and their possible practical significance is indicated.

Some earlier published work of the author (1) indicated a rather interesting inverse relationship between average air temperature just previous to harvest and amounts of total sugar in forages at or soon after harvest. Study of this relationship, expanded to include rainfall during the growing period and hours of bright sunshine just previous to harvest, forms the basis of this paper.

EXPERIMENTAL PROCEDURE

Seventy-two lots of forage including 20 hays, 37 early-cut green forages, and 15 green forages somewhat more mature have been included in the statistical analysis. The lots varied widely in botanical and chemical composition; a few were pure or nearly pure stands of alfalfa, red clover, or timothy; the great majority were mixed grasses and legumes, or mixed grasses. Sampling of all lots was done in the field soon after cutting, in the case of the green forages, and just before baling for the hay lots. All lots of hay included had been subjected to little or no rain after cutting.

Samples were taken to the laboratory immediately, where water determination was made by the Sterling-Bidwell method (2) of toluene distillation. The bulk of the sample was then dried rapidly in a large oven in a current of air thermostatically controlled at 80° C. Following drying, the samples were conditioned at room temperature for a week or more, ground in a Wiley mill to pass a ½-mm. sieve, and bottled for analysis. Sugar was determined by the Lane-Eynon method (3), results being expressed as amount of invert sugar in the dry matter.

Average content of total sugar was 6%, with a range from 1.4 to 13%. Forty-five of the samples were in the range between 4 and 7%, 60 of them between 4 and 10%. The meteorological records used for the study were those of

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the local weather station. Seasons included were 1951-1957. Average daily temperature was 65° F., ranging from 82.5° F. in August, 1957, to 49° F. in October of the same year (1957). Average length of the growing period was 43 days, with a range of from 16 to 73. Growing period was reckoned from April 30 for first cuttings, from the date of the previous cutting for those subsequent. Average amount of bright sunshine was 9.1 hr., ranging from 3.3 (June, 1951) to 11.7 hr. (August, 1956).

DISCUSSION OF RESULTS

The summary in Table 1 indicates that the only highly significant correlation was an inverse relationship between average air temperature and total sugar content of the forage (see Figure 1). Worthy of comment are:

1. The remarkably good agreement between all of the r values for total sugars *cf* average air temperature.

2. The slight cumulative effect of below-average temperatures on sugar content as revealed by increasing value of r in Column 3 of the table.

TABLE 1

Summary of correlation coefficients showing the relationship of certain weather phenomena to the amount of total sugars in forages as harvested

	All lots		Green forages only		Early-cut green forages	
Total sugars *Cf:*	d/f	r	d/f	r	d/f	r
Average air temperature						
During the 24 hr. previous to harvest	70	−.39**	50	−.42**		
During the 48 hr. previous to harvest		−.41**		−.43**		
During the week previous to harvest		−.46**		−.42**	35	−.40*
Rainfall during growing period	70	−.24*	50	−.25	35	−.30
Hours of bright sunshine	70					
On the day previous		.0003				
On the two days previous		.068				
During the week previous		.15				

* $P < .05$; ** $P < .01$.

3. Contrary to the original supposition, inclusion of results from 20 lots of hay and 15 lots of rather mature green forages did not significantly alter the values of r; what slight trend there was when these were included was to increase the r values. This situation was one factor in the decision to include these relatively mature crops along with the immature green forages. Average sugar content expressed as invert sugar in descending order was: immature green forages, 6.9%; all green forages, 6.5%; all lots, 6.0%; hays, 5.2%; late-cut green forages, 4.8%. Maturity appears as a definite influence here, but stage of maturity is a difficult thing to determine precisely. It was, therefore, decided that the greater statistical significance secured from the much larger number of samples warranted the inclusion of the data from the relatively mature lots of forage.

4. Rainfall during the growing season showed a consistent but not very significant inverse relationship to sugar content, regardless of whether the base for calculating r was 72, 52, or 37 lots.

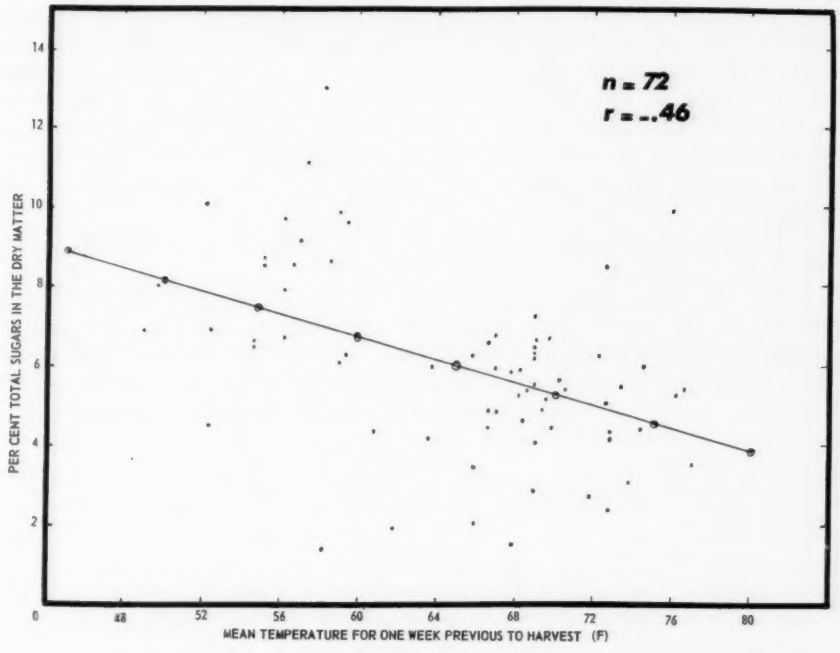

FIG. 1.

5. In general, sugar content of forages was not correlated with exposure to bright sunlight; however, when the basis of correlation was average hours of bright sunlight for a whole week previous to harvest, there was a slight but not significant relationship ($r = .15$).

These observations confirm our earlier work (1) and are in agreement with the work of Spoehr (8), who showed that high temperatures tend to decrease the monosaccharides of plants, while low temperatures tend to increase them. The general conclusion drawn from the reports of several other investigators is that strong light and high temperatures decelerate and under certain conditions inhibit photosynthesis. Wolff and Emmerie (11) state that with weak illumination a rise in temperature from 13 to 23° C. causes a decrease of about 60% in rate of CO_2 assimilation. Stolfelt (9) showed in studies with oats under field conditions that above certain limits light intensities and temperatures reduced photosynthesis. He gave the optimum temperature as 21° C., and stated (10) that assimilation rates of CO_2 show accommodation to low temperatures. A trio of Russian workers (12) noted that night temperature of 15° C. gave the best crop yield of tomatoes, 56% higher than when the plants were exposed to a constant temperature of 23°. Nekrasova (5) found more chlorophyll in young citrus leaves ". . . under conditions of weak light intensity and a short-day period than under conditions of strong light and longer-day period . . . More sugars are found in leaves exposed only for short-day periods in compari-

son with those exposed for long-day periods with either bright or diffused light." Kok (4) observed that ". . . the photosynthetic apparatus was destroyed by strong light." Noggle (6) states that ". . . as light intensity increases the curve flattens out and an increase in light intensity does not bring about a corresponding increase in the rate of photosynthesis—a situation known as light saturation." In view of all this, it is readily understood why exposure to bright sunlight showed little or no correlation with the sugar content of the forages.

The possible practical significance of these findings is pointed up by an as-yet-unpublished observation of the author, viz:

Relatively high sugar content of green forages is of some benefit in the making of good-quality grass silage. This work indicates that sugars tend to accumulate in forage crops during spells of relatively cool, dry weather. Farmers in a position to take advantage of such weather conditions may be able to produce better-quality silage than would be the case if the weather at ensiling time were relatively warm and humid. Such a situation may also account for the anomaly that good silage is sometimes produced without special precautions, whereas at other times, with the same kind of crop, and even on the same farm, failure results.

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RELATIONSHIPS BETWEEN AGE, BODY WEIGHT, AND YIELD OF DAIRY COWS[1]

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SUMMARY

Comparisons were made of the relative effects of age and weight on yield. Yield components studied were milk, milk fat, total solids, fat-corrected milk (FCM), solids-not-fat, protein, and lactose. There were 216, 305-day lactation periods of Holstein, Jersey, and Guernsey cows maintained as one experiment station herd. On an intra-breed basis FCM yield increased 570 lb/100 lb. in weight (significant), but when the effects of age were removed the increase was 236 lb. (not significant). Similarly, milk fat changes were 22 lb. (significant) and 9 lb. (not significant). On an intra-breed basis the relationship between yield and age was more than two times as important as weight as determined by the standard partial regression coefficients. Breeds for the various comparisons were strikingly different. Effects of weight on yield are interpreted too highly if differences due to breed and age are not removed.

Efficiency of milk energy yield by dairy cows is intimately associated with their metabolic capacity for converting feed energy into milk energy (14). Brody (5) called this dairy merit and defined it as the biological efficiency of milk production as measured by the percentage of consumed TDN energy which is converted into milk-energy (FCM). This definition of dairy merit (gross efficiency, lactation drive, etc.) is generally accepted and appears to have been first proposed by Gaines (14, 15). While the definition is straightforward and simple, practical application is essentially impossible. Production level is the essential component and this is related to size and heredity of the animals as well as to feeding practices and other environmental conditions.

When diverse populations of cows are used, it is clear that size and yield are positively correlated (9, 12, 16, 17, 19, 20, 23-25, 37, 38, 45, 46), particularly when age and breed are ignored (10, 16, 19, 26). Though larger animals produce more total milk energy than smaller animals, the yield per unit of weight favors the smaller animals. This is particularly evident when small species are compared with large species of animals (2, 4, 8). Brody (2-9), from his own voluminous work on bioenergetics and the work of others (14, 15, 18, 31-33), has generalized that larger animals move more slowly and move less than smaller animals and that this relationship can be described in terms of mass to surface area or in terms of resting maintenance requirements.

The 0.73 power of body weight ($W^{0.73}$) has been tentatively adopted as a reference base for energy metabolism (30) and Brody (5) suggested that $W^{0.7}$ describes the relationship between body weight and milk energy yield. This means that a 1,600-lb. cow, other things being equal, is expected to produce 1.70 times as much milk energy as an 800-lb. cow, rather than twice as much.

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From studies with rats, goats, and cows, Brody (4, 5, 8) reached two important conclusions: (a) If other conditions are equal, dairy merit is independent of body size and (b) milk-energy production, as maintenance cost, varies not with simple body weight, $W^{1.0}$, but with $W^{0.7}$, and that lactationally effective size is represented not by $W^{1.0}$ but by $W^{0.7}$. Kleiber (31, 32) was in general agreement. Later, Kleiber and Mead (33, 34) proposed that live weight be converted to metabolic size or $1{,}000 + (W - 1{,}000)^{0.75}$ and that dairy cows be evaluated on the basis of pounds of FCM per 1,000 lb. of metabolic size. Gaines (22) vigorously opposed the view that the relationship between live weight and milk-energy yield differed from $W^{1.0}$.

Though it has often been reported that the gross energetic efficiency of milk production is independent of body weight (2-5, 7, 21, 23, 25, 28, 29, 31-34), the criteria on which these interpretations were based were not the same. It is in this area that controversy existed (22, 34).

A practical question is whether, on an average, enough more yield is realized per unit increase in weight, other things being equal, to be more profitable to dairymen. Interest in this question was expressed over 60 yr. ago (47). Early investigators concluded that heavy cows produced more total milk, but that light cows produced more per 1,000 lb. of body weight (47, 48). Quantitative increases in milk fat production per 100-lb. increase in body weight has been estimated to be between 14-22 lb. (9, 36, 38, 43-46), where some statistical control of variable age was used. Similarly, FCM has been observed to increase between 200-250 lb. (12, 13, 16, 37).

The foregoing brief review of literature shows relatively good agreement among the several studies as they apply to greater efficiency in dairying. Admittedly, the data may best be reinterpreted giving more emphasis to breed differences and less emphasis to broad generalizations. With this purpose in mind, summaries on Washington State University dairy cows were made on the relationships between yield, body weight, and age.

EXPERIMENTAL PROCEDURE

From 1955 through 1959, the composition of the milk was determined at regular monthly intervals during 216 normal 305-day lactations of 154 cows (64 Holsteins, 44 Jerseys, and 46 Guernseys) in the University herd. Sixty-two cows had two consecutive records in the study. The cows averaged 44 mo. of age at calving and only seven were over 8 yr. old at the start of lactation. Only the Guernsey herd was handled in loose housing and had access to pasture for 4 to 6 wk. in late May and June each year. While on pasture, the Guernseys were fed alfalfa hay ad lib. Guernseys at other times, and Jerseys and Holsteins, were fed above-average-quality alfalfa hay and alfalfa-grass silage under dry-lot conditions. A 14% crude protein grain mix was fed according to the production of the cow and quality of roughage available. Grain-to-FCM ratios were generally wider than 1:4, since roughage quality was nearly always above average. The cows were milked twice daily. Body weights were taken between the 2nd and 6th mo. of lactation.

Daily milk weights were used, along with a 24-hr. composite, to determine the yields during the first 305 days following calving. The following assay methods were used: (a) Babcock for milk fat; (b) macro-Kjeldahl [(1), p. 12] for total protein, (c) Perry and Doan (40) for lactose; and (d) gravimetric for total solids, using a 5-ml. sample and 5 hr. of drying at 100° C.

Multiple regression and covariance techniques [(41), Chap. 14] were used for statistical evaluation. The variables were age at calving in months, weights of the cows, and the various components of yield in pounds. In addition to pounds of milk, the pounds of milk fat, protein, lactose, and total solids, solids-not-fat (SNF), and 4% fat-corrected milk (FCM) also were calculated. Weight was expressed in actual pounds and as metabolic size (33), except that the 0.7 power of weight ($W^{0.7}$) was used instead of $W^{0.75}$ as suggested by Kleiber and Mead (33). A 1,000-lb. cow was used as standard. Conversions to metabolic size were then $1{,}000 + (W - 1{,}000)^{0.7}$, where W is the body weight of the cow. The $W^{0.7}$ was used because earlier study of 200 of our experiment station cows gave an average relationship of $W^{0.69}$ (12), with 95% confidence limits between $W^{0.57}$ and $W^{0.81}$ (28). Though the breeds ranged from $W^{0.42}$ for Jerseys to $W^{1.14}$ for one Holstein herd, the slopes of the regressions were not significantly different ($P < 0.05$). The intra breed-herd regression was $W^{0.76}$, with 95% confidence limits between $W^{0.52}$ and $W^{1.0}$ (28).

RESULTS AND DISCUSSION

Metabolic size as defined above was carried through the analysis, along with actual weight. However, this alteration of weight description had no effect on the correlations as compared with actual weight. Though the magnitudes of the regressions in terms of metabolic size independent of age were increased for the Holsteins (12), the fractions due to regression and the relative importance of age and weight were unchanged. Therefore, in the following results, with the exception of Table 1, comparisons involving only actual weight are presented.

Lactation yields. Table 1 shows that the Holsteins on a per cow basis produced more milk, milk fat, FCM, SNF, protein, and lactose than Jerseys or Guernseys ($P < 0.005$). However, the Guernseys and Jerseys produced significantly more butterfat ($P < 0.01$) than the Holsteins per 1,000 lb. of cow. Similarly, there were only minor differences in average yield of FCM and protein among the three breeds, but the Holsteins produced significantly more milk, SNF, and lactose.

Average yields per 1,000 lb. of metabolic size, according to Brody (5) and Kleiber and Mead (33), would be a fairer comparison. On the basis of metabolic size as herein defined, it appears the Holsteins in this study were the most efficient producers of milk, FCM, SNF, protein, and lactose ($P < 0.005$), but lower in milk fat ($P < 0.005$). The general results in Table 1 show it is important to consider size when evaluating dairy merit (5). One does not have to make breed comparisons to illustrate the need for considering dairy merit, since individual variation in size of cows within breeds is larger than differences in breed averages.

TABLE 1

Average weight and yield during 305-day lactation yields

Breed	Records	Weight	Actual yields of					
			Milk	Fat	FCM	SNF	Protein	Lactose
	(No.)				*(lb.)*			
					Per cow			
Holstein	101	1,353	13,507	501	12,917	1,153	410	641
Jersey	57	928	7,125	404	8,914	665	268	345
Guernsey	58	1,015	8,428	421	9,688	760	287	409
Total	216	1,150	10,459	454	10,994	919	339	501
					Per 1,000 lb. of cow			
Holstein	101	1,353	10,000	371	9,560	854	303	476
Jersey	57	928	7,720	438	9,660	722	290	375
Guernsey	58	1,015	8,428	421	9,688	760	287	409
Total	216	1,150	8,990	402	9,627	795	295	432
					Per 1,000 lb. metabolic size (33)			
Holstein	101	1,235	10,920	405	10,450	933	331	520
Jersey	57	948	7,530	427	9,410	703	283	365
Guernsey	58	1,010	8,430	420	9,680	760	287	411
Total	216	1,099	9,360	415	9,970	826	306	446

Relative importance of age and weight. Table 2 shows the simple and partial linear regression coefficients and the fraction accounted for by the latter. Similarly, the correlations are in Table 3, which also shows the relative importance of age and weight as determined by the standard partial regression coefficients. Since previous workers have made comparisons involving milk fat or FCM, discussion of Tables 2 and 3 will be limited largely to these two measures of yield.

The simple regressions and correlations (Tables 2 and 3) clearly show the rather large values for the relationship of age or weight to yield when the effects of one are confounded with the other. These values are comparable with other similar data (10, 25, 26). On an intra-breed basis, a 100-lb. increase in weight (Table 3) showed a significant increase of 570 lb. FCM, but this was only 236 lb. for weight independent of age (not significant). In this latter case, age was more than two times as important as weight (Table 3).

Turner (44, 45) estimated weight to be 25 to 30% as important as age for Jerseys and Guernseys. Gaines (17), using a different procedure, reported that the effects of age and weight were about equal. Miller and McGilliard (37), using Michigan DHIA records, observed an intra-breed increase of 200 lb. of milk per 100-lb. increase in body weight, independent of age, for Holsteins, Jerseys, and Guernseys. Gaines (16) reworked McDowell's (36) 110,000 DHIA records and observed an increase of 250 lb. FCM per 100-lb. increase in size, but in this study age effects were confounded with weight effects. Turner (44, 45) observed increases of 18-22 lb. in butterfat production of Jerseys and Guernseys, using yearly age groups which, of course, partially removed the effects of age.

Two other studies involved weight and yield, but the records were first converted to a mature equivalent basis (13, 42). In one (13), Holsteins showed an increase of 16 lb. of milk fat per 100-lb. increase in weight. In the Jerseys (13), age conversion apparently accounted for all changes due to weight. The

TABLE 2

Changes in yield(y) per 12-mo. increase in age and 100-lb. increase in weight

Breed	Simple regression		Partial regression		Fraction due to regression—age, weight, and yield
	Age	Weight	Age	Weight	
	(lb.)				(R^2)
			Milk (y)		
Holstein	767	963	506	530	0.25
Jersey	340	369	338	−6[a]	0.31
Guernsey	2[a]	−361	207[a]	−516	0.00
Common	503	585	400	237[a]	0.15
Total	634	1,205	11[a]	1,201	0.53
			Fat (y)		
Holstein	27	32	20	15	0.19
Jersey	17	26	14	10	0.17
Guernsey	0[a]	−6[a]	3[a]	−9[a]	0.01
Common	19	22	15	9[a]	0.12
Total	21	23	12	19	0.23
			Total solids (y)		
Holstein	87	106	60	55[a]	0.21
Jersey	42	54	28	12[a]	0.15
Guernsey	−4[a]	−35[a]	14[a]	−45[a]	0.05
Common	56	67	44	29[a]	0.12
Total	70	115	12[a]	111	0.42
			FCM (y)		
Holstein	714	860	502	430[a]	0.21
Jersey	395	533	345	150[a]	0.18
Guernsey	−5[a]	−240[a]	127[a]	−336[a]	0.05
Common	487	570	384	236[a]	0.13
Total	570	824	177[a]	757	0.37
			SNF (y)		
Holstein	60	75	39	41[a]	0.21
Jersey	24	28[a]	24	2[a]	0.13
Guernsey	−4[a]	−29[a]	11[a]	−37	0.07
Common	38	45	29	20[a]	0.12
Total	48	93	0[a]	93	0.49
			Protein (y)		
Holstein	22	29	13	18	0.23
Jersey	10	13[a]	9	3[a]	0.14
Guernsey	0[a]	−5[a]	3[a]	−7[a]	0.02
Common	15	19	10	10	0.14
Total	18	30	3[a]	28	0.44
			Lactose (y)		
Holstein	30	38	21	20[a]	0.19
Jersey	13	12[a]	14	−4[a]	0.12
Guernsey	−4[a]	−22	7[a]	−27	0.11
Common	19	21	16	7[a]	0.10
Total	25	54	0[a]	48	0.44

[a] The regression is not significant at the 5% level of probability. In each comparison the slopes of the individual breed regressions are significantly different.

general data for increases in milk fat and FCM yields (Table 2), with increasing weight independent of age, are within the range of near-similar data reported by others (13, 37, 42, 44, 45).

Generally, Holsteins show a greater increase in yield as weight increases (10, 25, 28, Table 2) or as weight increases independent of age (10, 12, 13,

TABLE 3

Correlations (r) between yield (y) and age (x_1) and/or weight (x_2)

Breed	Simple (r)		Partial (r)		Relative importance of [a]	
	Age	Weight	Age	Weight	Age	Weight
	(coefficient)				*(ratio)*	
			Milk			
Holstein	0.46	0.44	0.26	0.24	0.30	0.24
Jersey	0.45	0.27	0.38	−0.01	0.46	−0.01
Guernsey	0.00	−0.26	0.22	−0.32	0.21	−0.38
Common	0.37	0.30	0.24	0.10	0.29	0.12
Total	0.31	0.73	0.01	0.69	0.01	0.73
			Fat			
Holstein	0.42	0.37	0.25	0.14	0.31	0.17
Jersey	0.40	0.32	0.27	0.11	0.32	0.13
Guernsey	−0.01	−0.10	0.05	−0.11	0.06	−0.13
Common	0.33	0.28	0.21	0.10	0.26	0.12
Total	0.34	0.46	0.18	0.37	0.19	0.37
			Total solids			
Holstein	0.43	0.40	0.24	0.17	0.30	0.21
Jersey	0.38	0.27	0.28	0.05	0.34	0.06
Guernsey	−0.03	−0.20	0.09	−0.21	0.11	−0.25
Common	0.33	0.28	0.21	0.10	0.26	0.12
Total	0.31	0.69	0.06	0.60	0.05	0.62
			FCM			
Holstein	0.44	0.40	0.26	0.17	0.31	0.20
Jersey	0.42	0.31	0.31	0.08	0.37	0.09
Guernsey	−0.01	−0.16	0.10	−0.16	0.12	−0.22
Common	0.35	0.29	0.23	0.10	0.28	0.12
Total	0.34	0.61	0.12	0.54	0.11	0.56
			SNF			
Holstein	0.43	0.41	0.24	0.19	0.28	0.22
Jersey	0.36	0.23	0.27	0.02	0.35	0.02
Guernsey	−0.05	−0.25	0.16	−0.26	0.13	−0.32
Common	0.33	0.28	0.21	0.10	0.26	0.12
Total	0.29	0.70	0.00	0.66	0.00	0.70
			Protein			
Holstein	0.43	0.43	0.22	0.22	0.26	0.26
Jersey	0.37	0.26	0.28	0.05	0.34	0.05
Guernsey	−0.06	−0.13	0.08	−0.11	0.09	−0.18
Common	0.34	0.32	0.20	0.15	0.24	0.17
Total	0.32	0.66	0.07	0.77	0.06	0.64
			Lactose			
Holstein	0.41	0.38	0.23	0.16	0.28	0.20
Jersey	0.34	0.17	0.30	−0.04	0.37	−0.05
Guernsey	−0.08	−0.31	0.11	−0.32	0.14	−0.39
Common	0.30	0.23	0.21	0.06	0.26	0.08
Total	0.26	0.70	−0.05	0.68	0.00	0.63

[a] Standard partial regression coefficients = by 1.2 $\sqrt{\Sigma x_1^2/\Sigma y^2}$ for age; by 2.1 $\sqrt{\Sigma x_2^2/\Sigma y^2}$ for weight.

Table 2), when compared with Ayrshires (10) or Jerseys or Guernseys. Tables 2 and 3 show that breed differences may be considerable and if cows of several breeds are compared as a single population, yield relationships with weight, independent of age, appear over five times as important as age.

The Guernseys involved in the present study show two undesirable features which are probably not generally characteristic of the breed. These are (a) less than expected increases in yield with aging and (b) the larger animals

were the poorer producers. In this regard, Davis *et al.* (10) observed a negative regression between yield of their Guernseys and weight independent of age, but Turner (44) found an 18-22 lb. increase in milk fat per 100-lb. increase in body weight within age intervals of one year. The results are disturbing, to the extent that they show what can happen in an individual herd if size is given undue emphasis without at the same time paying close attention to dairy merit (5).

Finally, Tables 2 and 3 show that yields of total solids, SNF, protein, and lactose show relationships similar to those already discussed for FCM and milk fat, though protein may be partially considered an exception. In the case of this component of milk, the relative importance of weight and age is more nearly equal (Table 3) on an intra-breed basis, i.e., near 40% for weight and 60% for age. Lactose on a similar basis is nearer 25% for weight and 75% for age. In this study the older cows secreted a lower percentage of lactose in the milk, thereby also lowering the percentage solids-not-fat. Protein percentage declined with age very little, if at all.

DISCUSSION

Over the years much controversy has occurred on the relative merits of age or weight correction. The present data are too few for generalizations. In fact, attempts to generalize the effects have been largely responsible for the more puzzling aspects of this general problem. Unquestionably, weight correction without regard to age would adjust at least partially for differences due to age, and the reverse is true. Neither the effects of age nor weight on yield are linear. When treated as linear, as in the present study, one can expect the estimates of their independent effects to be conservative. The total evidence as herein reviewed and discussed favors the view that yield does not increase proportionately with weight ($W^{1.0}$) but at a power of weight below 1.0, even when the effects of age are not removed. The literature is in relatively good agreement on the magnitude of increases in yield with each 100-lb. increase in weight, where near comparable methods of analysis were used.

Evaluation of cows on the basis of dairy merit (5) or efficiency (14, 15), or in terms of output/unit weight of cow (Table 1) appears necessary for improving their apparent feed efficiency. Touchberry (42), using mature equivalent records, observed no genetic correlation between weight and yield. However, Miller and McGilliard (37) studied the effects of age and weight independently and observed a genetic correlation of 0.3 between weight and yield. Turner (45) found that daughters increased 16 lb. in weight for each 100-lb. greater size among the dams.

The effects of increasing size on yield are sufficiently high that sires may well be labeled as outstanding transmitters of improved production which is entirely due to size and which may even result in a decrease in dairy merit. Under present methods of evaluating sires for ability to transmit increased production, increases as often as not may be due to increased size of the daughters as a result of inheritance and environment. If so, how much more should

these daughters produce to have dairy merit equal to smaller daughters of other sires?

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EFFECT OF INCREASED LEVELS OF OVARIAN HORMONES AND DURATION OF TREATMENT ON THE EXPERIMENTAL INDUCTION OF GROWTH OF THE COW'S UDDER [1, 2]

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SUMMARY

Mammary gland growth was induced experimentally by daily injection of 200 mg. of progesterone with 200 μg. of estradiol benzoate or by the feeding of 20 mg. of diethylstilbestrol daily for periods of 60, 120, and 180 days to 20 heifers and one steer. Lactation was then initiated by daily injections of 0.3 mg. of estradiol benzoate per 100 lb. body weight for 14 days. Treatment for 180 and 120 days apparently did not stimulate any greater mammary gland growth than 100 mg. of progesterone and 100 μg. of estradiol benzoate injected daily for 180 days, used in previous studies. The average maximum daily milk yields for the 16 animals on the lower level of hormones was 22.7 lb. per day, compared with the maximum daily milk yield of 23.8 and 21.2 lb. for the 14 animals treated for 180 and 120 days, respectively, with the higher level of estrogen and progesterone.

The mean maximum daily milk yield for the six heifers injected for 60 days was 10.2 lb. The average maximum daily milk yield for the three lowest producers was 3.9 lb., compared with 21.4 lb. per day for the three highest producers of this group.

Twenty milligrams of diethylstilbestrol administered orally proved equally as effective as 200 μg. of estradiol benzoate injected subcutaneously in synergizing with 200 mg. of progesterone injected daily to stimulate mammary gland development.

When the lactation curve of the experimental heifers began to decline after reaching their maximum milk yield, either estradiol benzoate at the level used to initiate lactation or the feeding of diethylstilbestrol at levels of 30 and 40 mg/day/100 lb. body weight was administered for seven days. During the week of administration, milk yield declined slightly. This was followed by a variable increase in milk yield for several weeks, reaching peaks of lactation, in some cases, above the initial maximum production.

Preliminary reports have been presented concerning the experimental induction of growth of the cow's udder and the initiation of milk secretion (3, 4). In these studies, 12 heifers were injected daily with a mixture of 100 μg. estradiol benzoate and 100 mg. of progesterone for 180 days. Four additional heifers were fed 10 mg. daily of diethylstilbestrol in place of the injected estrogen plus progesterone. Upon the subsequent induction of lactation with 3 mg. daily of estradiol benzoate for 2 wk., mean maximum daily FCM milk yield was 24.7 and 23.8 lb., respectively, for the two groups.

In continuing these studies, answers to several questions were sought. First, was the amount of ovarian hormones sufficient to stimulate the growth of the udders to the greatest extent possible? To answer this question, it was decided to double the level of the two ovarian hormones; namely, to 200 mg. progesterone and 200 μg. estradiol benzoate or 20 mg. diethylstilbestrol (orally).

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[2] Aided in part by a grant from the U. S. Atomic Energy Commission [Contract No. AT(11-1)-301]. Syntex Animal Products Company has generously contributed ovarian hormones.

Second, could the duration of the ovarian hormone injection period be reduced prior to the initiation of lactation? To answer this question, groups of heifers were injected for 60, 120, or 180 days.

Third, it seemed desirable to determine the oral effectiveness of diethylstilbestrol as compared to the injection of estradiol benzoate. In the present study, the diethylstilbestrol was fed at a level of 20 mg. daily.

Fourth, would estrogen which is effective in stimulating the initiation of lactation via the lactogenic hormone have an influence on milk yield after maximum production had been reached and milk secretion had begun to decline?

Finally, the influence of the administration of L-thyroxine at 50% above the winter secretion in late lactation was studied in one group of heifers.

EXPERIMENTAL METHODS

This experimental study of mammary gland growth in dairy cattle included 21 animals (Table 1). Daily subcutaneous injections of 2 mg. of the oil and benzyl alcohol solution containing 200 mg. progesterone or 200 mg. of proges-

TABLE 1

Data on experimental cattle

Breed & herd No.	Duration of hormone treatment *(days)*	Experimental growth of udder started	Age at initiation of lactation *(mo.)*	Body weight		Remarks
				At start *(lb.)*	At end *(lb.)*	
Group I						
BS 26	180[a]	October 2, 1958	25	940	1,245	Normal
BS 27	180	October 2, 1958	20	810	977	Normal
J 594	180[a]	October 2, 1958	23	585	787	Normal
J 631	180	October 2, 1958	29	775	982	Normal
H 863	180	October 2, 1958	26	865	1,190	Normal
H 868	180[a]	October 2, 1958	23	820	1,102	Normal
H 872	180	October 2, 1958	21	760	1,015	Normal
Group II						
BS 24	120	October 2, 1958	26	1,000	1,205	Normal
BS 25	120[a]	October 2, 1958	26	910	1,040	Normal
G 478	120[a]	October 2, 1958	38	1,085	1,190	Sterile
J 630	120	October 2, 1958	29	805	970	Normal
H 869	120[a]	October 2, 1958	21	865	1,060	Normal
H 870	120[a]	October 2, 1958	21	820	970	Normal
H 873	120	October 2, 1958	18	775	900	Normal
Group III						
H 879	60	October 25, 1959	24	980	1,020	Normal
H 263	60	October 25, 1959	25	835	920	Normal
H 265	60	October 25, 1959	24	865	945	Normal
J 635	60	October 25, 1959	27	590	630	Grade rumen fistula sutured at start of experiment
J 644	60	October 25, 1959	33	870	910	Normal
G 261	60	October 25, 1959	25	840	930	Normal
H 266	180	February 14, 1959	24	625	980	Steer

[a] Received 20 mg/day of diethylstilbestrol orally in place of 200 μg/day of estradiol benzoate.

terone and 200 μg. of estradiol benzoate were administered throughout the growth phase.

The diethylstilbestrol was prepared with celluflour and premixed with finely ground soybean meal, then mixed with the concentrate feed. One pound of feed containing 20 mg. of diethylstilbestrol was fed once a day before the regular feeding.

As animals became available they were placed in three groups for the mammary gland growth study. Group I consisted of seven heifers, which were treated for 180 days. Group II included seven heifers which were injected for 120 days. Group III included six heifers that received progesterone and estradiol benzoate for 60 days. To compare oral effectiveness of diethylstilbestrol with the injection of 200 μg. of estradiol benzoate in synergizing with 200 mg. progesterone to stimulate mammary gland growth, three heifers from Group I and four heifers from Group II were fed 20 mg. of diethylstilbestrol daily to replace the estradiol benzoate. The remaining animals in each group received progesterone in combination with estradiol benzoate by injection.

The animals in Group I received increased estrogen the last 30 days of the growth phase. The estradiol benzoate was increased to 1 mg. the first ten days to 2 mg. the second and to 3 mg. the last ten days of this period.

To initiate lactation at the end of the growth phase, progesterone injections were discontinued and 0.3 mg. of estradiol benzoate per 100 lb. body weight was injected daily for 14 days. Milking was commenced 12 hr. after the first injection and continued twice daily for the duration of the experiment.

When the heifers began to decline in milk production for periods of 1 to 3 wk. after reaching maximum production, estrogen treatment was again tried during a period of 1 wk. The heifers in Group I were treated orally with 30 mg. of diethylstilbestrol/100 lb. body weight daily during the 10th wk., 40 mg. daily during the 17th wk., and injected with 0.3 mg. estradiol benzoate/100 lb. body weight during the 25th wk. of lactation.

Animals in Group II were injected with 0.3 mg. of estradiol benzoate/100 lb. body weight during the 9th wk. of lactation and Group III the same amount during the 9th or 10th wk.

Group II was injected with L-thyroxine beginning Week 24 for 8 wk. at a level 50% above their winter thyroxine secretion rate.

EXPERIMENTAL RESULTS

A noticeable lengthening of the tests occurred after 3 to 4 wk. of hormone therapy. The skin of the udder and the vulva showed some increased hyperemia, whereas external appearance of the udders indicated little or no growth at the end of estrogen-progesterone treatment; however, rapid enlargement occurred after the initiation of estradiol benzoate administration.

The two heifers, No. 872 from Group I treated with progesterone and estradiol benzoate, and No. 478 of Group II which received progesterone and diethylstilbestrol, showed signs of estrus 87 and 112 days after the start of hormone treatment. All heifers showed marked edema of subcutaneous tissue of the

udder starting when estradiol was injected to initiate milk secretion, with the exception of the three low-producing heifers in Group III. This condition persisted throughout the period of estrogen treatment, but disappeared within a few days after discontinuing the estrogen injections. In several of these animals the edematous condition extended from the udder to the navel area. Increased hyperemia and swelling of the vulva occurred concurrent with the udder edema. Slight to considerable relaxing of the sacroiliac and sacrosciatic ligaments, causing a sinking in front of the tuber eschii (pin bones), as is noticed in cows a few hours before parturition, occurred during the estrogen treatment.

The seven heifers which were treated for 180 days with progesterone and estrogen were injected with increasing amounts of estrogen during the last 30 days of the growth phase, to determine if milk secretion would be initiated gradually, as is believed to occur during the last one-third of pregnancy. Only two of the seven cows (No. 27 and No. 631) showed signs of bagging up during this period. When progesterone was stopped, their increase in milk yield was no more rapid than the others in the group when estradiol benzoate alone was administered. The mammary secretion on the first day after estrogen administration ranged from 6 to 70 ml., consisting of a sticky, viscous, honey-colored material changing to a colostral appearance as yields increased to 200 to 400 ml. per day. The secretion gradually changed to normal-appearing milk as daily production reached 4 to 10 lb. per day. Rapid increases in milk yields occurred from the third to the ninth day in this group. The mean daily production, computed as the average of a seven-day period, increased from 0.1, 0.3, 3.0, 2.8, 2.0, 0.09, and 0.4 lb. in the 1st wk. to 20.5, 17.1, 20.1, 24.7, 30.9, 28.6, and 28.7 lb. in 6 to 9 wk. for heifers numbered 26, 27, 594, 631, 868, 863, and 872, respectively. The mean maximum daily milk yield of 23.8 lb. per day for this group was reached in the 6th wk. (Figure 1). No difference in milk yield was observed due to the estrogen injection compared with diethylstilbestrol fed with progesterone to stimulate mammary gland growth. Animals in this group which received 20 mg. of diethylstilbestrol orally reached a mean maximum daily production of 23.6 lb. per day in the 6th wk., compared with a peak of 24.0 lb. per day at the same time for heifers which were injected with 200 μg. of estradiol benzoate.

In Group II, as in all three groups, milking was commenced 12 hr. after the injection of estradiol benzoate alone was started. The first secretion and the change to normal-appearing milk was the same in all three groups. The mammary secretion obtained from this group on the first day ranged from none to 65 ml.; no secretion was obtained from No. 873 on the first two days after milking was started. The mean daily production (based on a weekly average) ranged from 0.36, 1.6, 1.6, 1.1, 0.7, 2.7, and 0.8 lb. the 1st wk., reaching a peak daily production of 20.8, 20.9, 23.6, 23.4, 21.2, 21.5, and 20.7 lb. in 7 to 9 wk. for animals numbered 869, 478, 25, 24, 870, 630, and 873, respectively. The average maximum daily milk yield for Group II was 21.2 lb. per day in the 7th wk. (Figure 2). This was slightly lower than Group I; however, not significantly different at the 5% level. The mean daily maximum production of the heifers

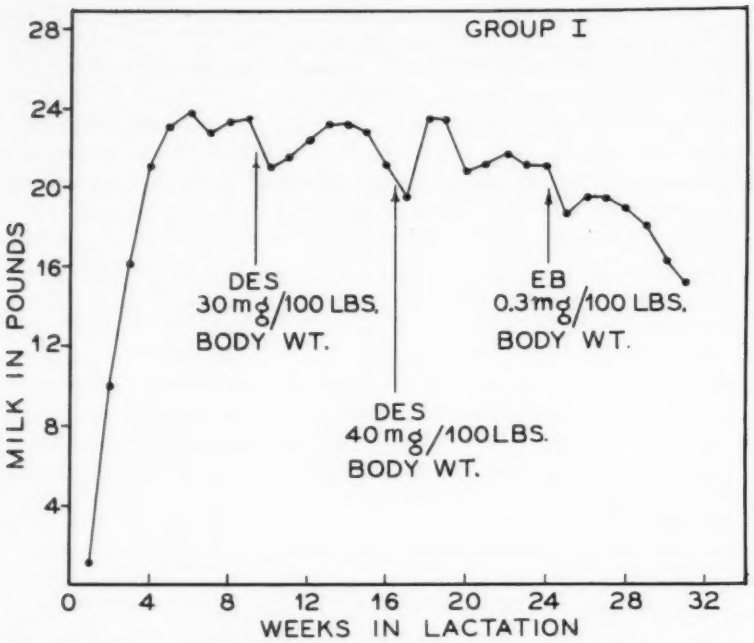

FIG. 1. Mean daily milk yield for weekly periods for Group I, 180-day growth phase. DES designates diethylstilbestrol, fed for 1 wk. EB denotes estradiol benzoate injected for seven days.

fed diethylstilbestrol was 21.1 compared with 21.3 lb. per day for the animals of this group which were injected with estradiol benzoate to synergize with progesterone to stimulate mammary gland growth.

The six heifers treated with progesterone and estrogen for 60 days showed much greater variation in milk yield. The total production for the first seven days ranged from a few milliliters to 2.6 lb., reaching a maximum daily milk yield (weekly average) of 26.1, 23.8, 16.7, 6.2, 3.6, and 3.3 lb. for heifers numbered 879, 261, 644, 635, 265, and 263, respectively, in 4 to 8 wk. The mean maximum daily milk yield of 21.4 lb. per day for the three higher-producing animals in Group Three was comparable to the peak yield of the animals treated for 180 and 120 days. The average peak yield of the three lower-producing heifers, however, was only 3.9 lb. per day. The maximum daily production was reached at the 4th wk. by the low producers, whereas the peak production was attained in the 7th wk. by the higher producers (Figure 3). The mean daily yield of 10.4 lb. for Group III was considerably less than that of the two other groups.

An evaluation of the differences in the extent of mammary gland growth stimulated during the three treatment periods was obtained, using the lactation index as a measure of udder growth (Table 2). The difference was tested by

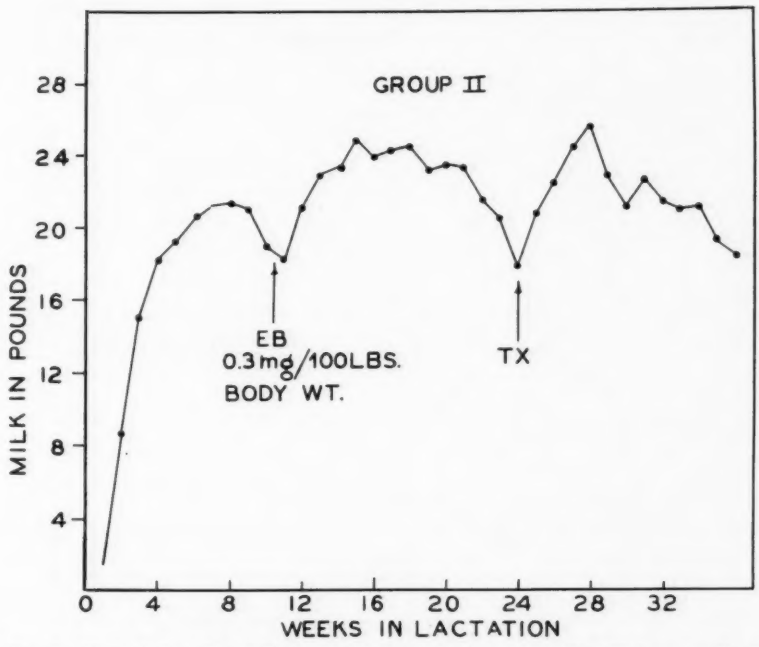

FIG. 2. Mean daily milk yield for weekly periods for Group II, 120-day growth phase. EB designates estradiol benzoate injected for 1 wk. TX denotes L-thyroxine injected daily for 12 wk. at 50% above winter secretion rates.

the "t" test. There was no significant difference between the mean maximum daily milk yield of Group I and Group II during the first 8 wk. after the initiation of lactation or during the next 8 wk. following injections of 0.3 mg. of estradiol benzoate or by feeding 30 mg. of diethylstilbestrol per 100 lb. body weight daily for seven days following a decline in production for 1- to 3-wk. periods ($P > .05$). Mammary gland growth stimulated during the 60-day injection period was significantly less than that of the two other groups treated

TABLE 2

Comparison of milk yield for the three treatment periods

Total pounds of FCM produced								
180-day treatment			120-day treatment			60-day treatment		
Herd No.	8 wk.	16 wk.	Herd No.	8 wk.	16 wk.	Herd No.	8 wk.	16 wk.
594	1,168.6	2,506.1	24	1,096.8	2,521.2	263	121.3*	196.7
868	1,306.4	2,802.6	25	988.5	2,369.8	265	136.9*	350.7
863	1,023.6	2,104.9	630	882.7	2,547.0	635	296.3*	852.8
872	1,243.0	2,561.0	478	1,006.8	2,165.9	879	1,102.9	2,320.7
631	1,363.9	3,019.0	873	872.3	2,155.7	261	1,028.2	2,153.8
26	822.2	2,040.5	870	908.9	2,120.5	644	845.7	2,178.7
27	789.8	1,642.0	869	846.4	1,833.9			
Mean	1,113.6	2,393.9		975.7	2,244.9		589.9*	1,342.2

* Significantly different from the mean of the 180-day treated group ($P > .05$).

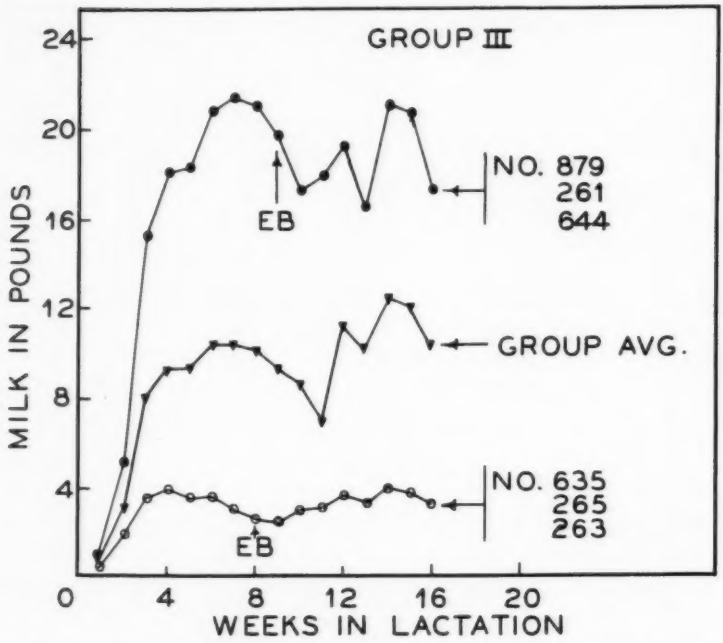

FIG. 3. Mean daily milk yield for weekly periods for Group III, 60-day growth phase. EB designates estradiol benzoate injected for 1 wk.

for 180 and 120 days ($P > .05$). However, the average of the three high-producing animals in Group III was not significantly different from either Group I or Group II ($P > .05$).

One Holstein steer numbered 266 was treated with 200 mg. of progesterone and 200 μg. of estradiol benzoate daily for 180 days to stimulate mammary gland growth. Three weeks after the initial injection of the ovarian hormones, increases in diameter and length of the teats were observed. By the end of the 5th mo. a whitish fluid very much like milk could be obtained. A maximum of 15 ml. per day was secreted 2 wk. after the estrogen treatment. A second 14-day estradiol benzoate injection period did not stimulate increased secretion.

Richardson (1) suggested that the difficulty in obtaining full mammary gland development in male cattle is due to a poorly developed inguinal fatty pad in these animals. Turner (2) noted a lack of udder growth in fetal development of the male bovine. As a result of a lack of udder development, the mammary gland lacks space in which to grow except at the base of the teat. In contrast, in the female the udder consists of a fatty pad in which the ducts and lobule-alveolar system can grow extensively under the stimulus of suitable hormones.

Three heifers from Group I and four from Group II were fed 20 mg. of diethylstilbestrol daily in place of 200 μg. of estradiol benzoate injected with 200 mg. of progesterone to stimulate mammary gland growth. The mean total

TABLE 3
Effect of oral or injected estrogen with 200 mg. injected progesterone on milk yield

Total pounds of FCM produced					
20 mg. diethylstilbestrol orally			200 μg estradiol benzoate injected		
Herd No.	8 wk.	16 wk.	Herd No.	8 wk.	16 wk.
594[a]	1,168.6	2,506.1	24	1,096.8	2,521.2
868[a]	1,306.4	2,802.6	27[a]	789.8	1,642.0
870	908.9	2,120.5	630	882.7	2,547.0
869	846.4	1,833.9	631[a]	1,363.9	3,010.0
478	1,006.8	2,165.9	872[a]	1,243.0	2,561.0
26[a]	822.2	2,045.0	873	872.3	2,155.7
25	988.5	2,369.8	863[a]	1,023.6	2,104.9
Mean	1,016.6	2,262.8		1,071.4	2,375.9

[a] These animals were treated for 180 days. The other animals in each group were treated for 120 days. There was no significant difference in the mean production between the two treatments ($P > .05$).

production at 8 wk. of 1,016.6 lb. of milk for the seven animals administered diethylstilbestrol was compared to the mean of 1,071.4 lb. for the seven heifers injected with estradiol benzoate (Table 3). The difference was tested by the "t" test. There was no significant difference in the mean production between the two treatments, either at the peak reached in 8 wk. after the initiation of lactation or in 16 wk. after subsequent treatment with 0.3 mg. of estradiol benzoate per 100 lb. body weight injected to Group II during the 10th wk. or feeding 30 mg. of diethylstilbestrol per 100 lb. body weight daily to Group I during the 9th wk. of lactation ($P > .05$).

When three animals in Group III lactated so well after an injection period of 60 days, it was thought of interest to determine the effect of the estrogen treatment alone. Two 26-mo.-old Jersey heifers were available. They were injected with estradiol benzoate at a level of 3 mg/day for 14 days, similarly to the others. A maximum average daily milk production of 15.5 and 13.9 lb. per day was reached during the 9th wk. following the start of the injection of estrogen.

Comparison of productivity of experimental animals with relatives. An estimate of the extent of mammary gland development in the experimental heifers may be obtained by the comparisons of the maximum daily milk yields of related animals. Comparison of the mean maximum daily production (based on a weekly average) of maternal and paternal half-sisters with that of animals in Group I showed that the experimental animals produced from 61.3 to 108.4% of the mean of the related animals lactating after normal pregnancy. It will be noted from the range in production that some experimental animals exceeded the production of some of their lower producing half-sisters (Table 4). Similarly, in Group II, the comparison ranged from 55.8 to 135.8%. Since only three animals in Group III produced approximately normal milk yield, only individual comparisons are of significance.

Effect of estrogen upon established lactation. Since estrogen has been effective in these studies in initiating lactation and in causing milk secretion to increase for many weeks, it was decided to determine the influence of this hormone after

milk yield had reached a peak and had begun to decline. The administration of estrogen to the heifers in all three groups showed a decline in milk yield during the week of estrogen treatment. This was followed by an increase during the following 1 to 3 wk., up to, or considerably above, the level of production expected.

In Group I, the administration of two levels of diethylstilbestrol orally caused an initial dip in production, with a subsequent rebound to a higher level (Figure 1). In Group II the injection of estrogen during the 9th wk. stimulated an increase from 19.0 to 24.9 lb. per day, an increase of 31.1%. This production was considerably above the mean maximum milk yield previously attained by this group.

These data indicate the desirability of determining whether cows after normal parturition might respond favorably to a short period of estrogen when they begin to decline in milk production.

Effect of thyroxine on milk secretion. The winter thyroxine secretion rate of each heifer in Group II was determined. On the 24th wk. of lactation, L-thyroxine was administered daily to each animal at a level 50% above their secretion rate and was continued for 8 wk. It will be noted that milk yield increased rapidly for a period of 4 wk, reaching a peak of milk yield higher than had been reached previously by this group. While milk yield then began to decline, the milk yield at the end of the experiment was still above the mean yield at the time thyroxine was administered (Figure 2). These data indicate

TABLE 4

Comparisons of maximum daily milk yield of experimental animals with related animals

	Comparison of related animals			Experimental animals	
Herd No.	Type of comparison	Maximum milk production (lb/day)	(Range)	Maximum milk production (lb/day)	Comparison (%)
Group I					
863	11 Half-sisters	61.5	48.8–74.8	37.7	61.3
868	14 Half-sisters	51.4	42.0–62.2	40.9	79.6
872	14 Half-sisters	51.4	42.0–62.2	39.6	77.0
26	2 Half-sisters	32.1	19.6–46.1	34.8	108.4
27	1 Full sister	36.2		25.6	70.7
631	12 Half-sisters	32.3	24.1–39.9	32.0	99.1
594	12 Half-sisters	32.3	24.1–39.9	27	83.6
Group II					
869	11 Half-sisters	61.5	48.8–74.8	34.3	55.8
870	14 Half-sisters	51.4	42.0–62.2	42.2	82.1
873	14 Half-sisters	51.4	42.0–62.2	37.9	73.7
478	16 Half-sisters	38.9	29.6–58.7	23.4	60.2
24	2 Half-sisters	32.9	19.6–46.1	35.6	108.2
25	2 Half-sisters	32.9	19.6–46.1	44.8	135.8
630	12 Half-sisters	32.3	24.1–39.9	27.6	86.3
Group III					
879	14 Half-sisters	51.4	42.0–62.2	33.9	66.0
644	12 Half-sisters	32.1	19.6–46.1	20.2	62.5
261	Record of dam	32.6		29.3	89.9
265	Record of dam	39.4		4.4	11.2
263	Record of dam	63.9		5.9	9.9
635	Grade—No records available for comparison				

that the thyroxine secretion rate of these animals was limiting their capacity to secrete milk.

DISCUSSION

The present data indicate that 200 mg. progesterone and 200 μg. estradiol benzoate (ratio 1:1,000) stimulated no greater growth of the udders of these heifers (as indicated by the lactation index) than did 100 mg. progesterone and 100 μg. estradiol benzoate (1:1,000) used in the previous studies (3, 4). At the lower level, the mean maximum daily milk yield for 16 animals of 22.7 lb/day compares quite well with the peak yields of 23.8 and 21.2 lb/day reached by animals of Group I and II following injection periods of 180 and 120 days, respectively. While greater growth of the mammary gland may be stimulated by the use of other ratios or changes in the plan of administration, such as increasing or decreasing amounts of the two hormones, it would appear that 100 mg. progesterone and 100 μg. estradiol benzoate is optimal at ratios of 1:1,000 of the two hormones by uniform administration.

The average maximum daily milk yield of Group II treated with progesterone and estrogen for 120 days reached a peak of 21.8 lb. in 7 wk., whereas Group I treated for 180 days reached an average peak yield of 23.8 lb. per day in 6 wk. Following comparable estrogen treatment during the 9th or 10th wk., the maximum average daily yield of the group with the shorter treatment period reached 24.8 lb. during the 15th wk., whereas the peak of 23.2 lb. per day for Group I was reached in the 14th wk. There was no significant difference in the mammary gland growth stimulated in 180 as compared to 120 days of administering 200 mg. of progesterone and 200 μg. of estradiol benzoate.

The importance of progesterone in stimulating lobule-alveolar growth in dairy cattle has been questioned by some investigators, because estrogen alone stimulates variable amounts of milk secretion. In the light of the current research, where the udders were stimulated with estrogen and progesterone for 60 days, it would appear that those animals which are capable of secreting approximately normal amounts of milk either are capable of responding very rapidly to the gonad hormones or have developed more secretory tissue during recurring estrous cycles than had been believed possible heretofore. The latter suggestion is believed to be most reasonable. If this explanation is true, the recurrence of the estrous cycle after reaching sexual maturity, with the secretion of estrogen and progesterone, is capable of stimulating not only the growth of an extensive duct system in the udder but of lobule-alveolar growth as well. Thus, heifers which have not been bred for a year or more after sexual maturity has been attained may have sufficient lobule-alveolar growth to respond to the administration of estrogen by considerable yields of milk. However, not all heifers appear to respond to the gonad hormones secreted by recurring estrous cycles. Thus, in Group III one might suggest that the 2-mo. injection period was effective in continuing udder growth in three animals, whereas in the others little or no lobule-alveolar growth was present at the time hormone injections were started. The explanation of these differences in heifers in their capacity to respond to the hormones of recurring estrous cycles will be sought. They

may be deficient in secretion of other hormones which synergize with the gonad hormones in stimulating mammary gland growth.

With a limited number of animals, Turner (3) found that 10 mg. of diethylstilbestrol fed daily synergized as well with a 100 mg/day injection of progesterone as a 100 μg/day injection of estradiol benzoate in stimulating mammary gland growth in cattle. The udder growth stimulated by 20 mg. of orally administered diethylstilbestrol and 200 mg. of progesterone was equal to that obtained by daily injections of 200 μg. of estradiol benzoate plus 200 mg. of progesteone in both groups of heifers (Table 3). This agreement with previous work indicates that 1 mg. of diethylstilbestrol administered orally is equally as effective as 10 μg. of estradiol benzoate injected subcutaneously in synergizing with progesterone to stimulate mammary gland growth.

In a previous study (5), the feeding of 10 mg. of diethylstilbestrol daily during a period of 4 wk. tended to arrest the decline in milk yield and in some animals caused a slight increase. In the experiments reported here, the estrogen treatment was more intense and of 1-wk. duration. In the case of estradiol benzoate, the use of 3 mg/100 lb. body weight was the same as that used to initiate lactation following the growth phase of development. Concerning diethylstilbestrol, no previous experience in its use for this purpose was available. The use of 30-40 mg/100 lb. body weight in Group I was not as marked as the use of 3 mg. estradiol in Group II following the peak of lactation. Whether this was due to the level of diethylstilbestrol or to differences in the two groups of cattle is not known.

At any rate, in Group II, the response to estradiol was marked and stimulated an increase in milk yield above the peak of production reached following the initial stimulation. The observations suggest that the lactogenic hormone secretion may again be stimulated during lactation, as well as initially by estrogen. It is possible that normal lactating cattle might respond similarly to estrogen when they begin to decline after reaching maximum production.

In Group II, the favorable influence of thyroxine in stimulating increased milk secretion even in late lactation was shown. The peak of milk yield for the lactation was reached in the 28th wk. of period. It is clear that these heifers were secreting less than optimal amounts of thyroxine for, upon the injection of 50% above their secretion rates, milk yield was stimulated markedly.

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HERITABILITY AND REPEATABILITY OF FIRST, SECOND, THIRD, AND FOURTH RECORDS OF VARYING DURATION IN BROWN SWISS CATTLE [1,2,3]

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SUMMARY

Heritability estimates of first, second, third, and fourth lactations, averages of two, three, and four records, and of 100- and 200-day partial first lactations, were made to study the reliability of using partial or complete first records as the basis for early selection in Brown Swiss cattle. The data consisted of 8,413 HIR records of not less than 200 or more than 305 days in length from 38 Brown Swiss herds in Wisconsin.

Heritability estimates, obtained by doubling the intrasire regression of daughters on dams, were 0.42 for milk, 0.37 for butterfat, and 0.80 for percentage of butterfat for the first lactation. Estimates for milk and butterfat production, using partial first records, were of the order of 0.30 and 0.34 for 100- and 200-day yields, respectively. For percentage of butterfat, the values were 0.44 and 0.63, respectively. Repeatability values were 0.47 for milk, 0.45 for butterfat, and 0.68 for percentage of butterfat. Herds, sires, and cows in sire accounted for 25, 14, and 21%, respectively, of the total variance in milk yield.

In selecting for production traits in Brown Swiss cattle, first records appear to be as valuable as any other single record or average of records. Partial first records of 100 or 200 days in length will be almost as effective in improving milk and fat yield as selection based on 305-day first records.

The heritability estimates found in this study of Brown Swiss cattle are slightly higher than those previously reported for production traits in other breeds of dairy cattle in the United States. However, most of the previously reported estimates fall within the lower range of the fiducial limits reported here.

The reliability of the first record as the basis for early selection in the dairy cattle breeding program is important. Several workers (1, 2, 5, 16, 18) have felt that an average of two or more records is needed in order to have a reliable indication of an animal's producing ability. Others (4, 9) report that the highest record of a cow is a better index of her transmitting ability for production than is the average of records. Heizer (11), in 1933, and more recent studies (12, 13, 22, 24), have shown that selection based on the first record may be fully as reliable as where additional records are included. Johansson (12) concluded that the first record is superior to the second and slightly superior to the third as an indication of the cow's inherent capacity for yield.

Partial records may be helpful in making an early evaluation for selection. Several workers (6, 8, 20, 21, 23, 27) have reported on the reliability of using short records. Madden *et al.* (21) have developed factors for extending short records on Holstein-Friesian cattle.

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[2] Approved for publication by the Director of the Experiment Station, University of Wisconsin, Madison.

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A knowledge of the heritability of production traits is important for the dairy cattle breeder. While the heritability of milk and butterfat yield has been estimated by several workers, there has been little extensive study of Brown Swiss cattle. Lush and Straus, (19) using 269 daughter-dam pairs, found the heritability of butterfat yield to be 0.17. No other estimates of heritability and no repeatability values are available in the literature, although two reports (25, 28) provide background information for the breed.

In the present study, heritability estimates of first, second, third, and fourth lactations, averages of two, three, and four records, and of 100- and 200-day partial first lactations, were made to study the reliability of using partial or complete first-lactation records as the basis for early selection in Brown Swiss cattle. Repeatability estimates were also computed, since these values are lacking for the Brown Swiss breed.

DATA AND METHODS

The data consisted of 8,413 lactation records of not less than 200 or more than 305 days in length from 38 Brown Swiss herds in Wisconsin. These herds had been on HIR test for five or more consecutive years between 1936 and 1957. Original monthly entries were obtained from the Brown Swiss Cattle Breeders Association at Beloit, Wisconsin, and converted to punched cards. The 305-, 200-, and 100-day records were computed according to regular DHIA procedure and converted to a mature-equivalent, 2× basis, using the Brown Swiss factors of Kendrick (15). Only those cows having first-lactation records beginning at 36 mo. of age or less were used in the study. There were 1,764, 1,245, 844, and 560 cows having first, second, third, and fourth records, respectively.

Daughters and dams were paired according to each of their first four lactations. The records of dams having more than one daughter were repeated for each daughter. Heritability estimates for milk, fat, and percentage of fat were obtained by doubling the intrasire regression of daughter on dam. Repeatabilities were computed as intra-class correlations (14, 17, 26) by using variance components from the analysis of variance. Only the sires having at least two daughters with four records each were used in determining repeatability. A total of 1,760 records, 440 cows and 87 sires, met these requirements.

RESULTS AND DISCUSSION

The averages and standard deviations for milk, fat, and percentage of fat for each of the lactation groups of dams and daughters are shown in Table 1. A general appraisal of the means and standard deviations in all first-record cows vs. first-record cows held for later records indicates that these pairs have been subjected to a limited amount of selection for milk and butterfat yield. It is apparent that some culling had occurred in these herds before the fourth and fifth lactation.

The heritability estimates for the various records are shown in Table 2. The lactation groups include (a) all first records of which there were 646 daughter-dam pairs involving first records,(b) cows with two records (382 daughter-dam

TABLE 1

Averages and standard deviations of milk, fat, and percentage of fat yield of daughters (y) and dams (x) for various lactation groups

Lactation group [a]	No. of pairs	Milk (lb.)				Fat (lb.)				Fat (%)			
		$\bar{y}_1$	σy_1	$\bar{x}_1$	σx_1	$\bar{y}_2$	σy_2	$\bar{x}_2$	σx_2	$\bar{y}_3$	σy_3	$\bar{x}_3$	σx_3
1st (all)	646	10,458	2,459	10,410	2,246	433	100	426	90	4.16	0.32	4.09	0.33
1st of 2	382	10,691	2,270	10,510	2,182	442	92	427	85	4.15	0.33	4.08	0.33
1st of 3	210	10,806	2,141	10,660	2,210	448	88	433	88	4.16	0.33	4.08	0.34
1st of 4	104	11,219	2,126	10,840	2,194	464	86	440	86	4.15	0.34	4.07	0.34
2nd (all)	382	10,190	2,500	10,510	2,360	422	106	426	92	4.15	0.33	4.07	0.33
2nd of 3	210	10,555	2.280	10,570	2,339	439	98	428	92	4.16	0.33	4.07	0.34
2nd of 4	104	11,021	2,250	10,710	2,266	457	95	434	89	4.16	0.32	4.08	0.34
3rd (all)	210	10,647	2,662	10,560	2,511	441	117	427	98	4.14	0.35	4.07	0.35
3rd of 4	104	11,091	2,577	10,700	2,642	459	110	432	101	4.15	0.35	4.06	0.34
4th (all)	104	10,715	2,916	10,650	2,651	439	119	424	97	4.11	0.35	4.02	0.35

[a] First of two refers to first-record daughter-dam pairs where each have two records, etc.

TABLE 2

Heritability estimates of first, second, third, and fourth records and of averages of records

Lactation [a]	Heritability		
	Milk	Butterfat	Percentage of butterfat
All first records	0.42**	0.37**	0.80**
Cows with two records			
First record	0.26*	0.28*	0.55**
Second record	0.30**	0.38**	0.69**
Cows with three records			
First record	0.33*	0.35*	0.62**
Second record	0.22	0.22	0.80**
Third record	0.33*	0.16	0.70**
Cows with four records			
First record	0.42*	0.47*	0.53**
Second record	−0.10	−0.07	2.35**
Third record	0.11	−0.09	0.75**
Fourth record	0.58**	0.50*	0.71**
Averages of records of both daughter and dam			
Frist two records	0.36**	0.39**	0.68**
First three records	0.37**	0.25**	0.86**
First four records	0.40*	0.32	0.87**
First record of daughter and average of:			
First two records of dam	0.34**	0.35**	0.68**
First three records of dam	0.42**	0.36*	0.81**
First four records of dam	0.50*	0.56*	0.78**

[a] There were 646 daughter-dam pairs with one record; 382 pairs with two records; 210 pairs with three records, and 104 pairs with four records.

* Significant at the 5% level of probability.

** Significant at the 1% level of probability.

pairs), (c) cows with three records (210 daughter-dam pairs), (d) cows with four records (104 daughter-dam pairs), (e) average of the first two, three, and four records of both daughters and dams, and (f) the first records of daughters and the average of the first two, three, and four records of dams.

Using all first-record pairs, heritability estimates of 0.42, 0.37, and 0.80 were obtained for milk, fat, and percentage of fat, respectively. The corresponding regression coefficients and standard errors were $0.211 \pm .041$, $0.188 \pm .043$, and $0.402 \pm .037$. These estimates were, with few exceptions, greater than those found using any other single record or average of records, and are in general agreement with the work by Johansson (12) and Rendel *et al.* (23). Considerable variation and rather high standard errors (0.05 to 0.10) were found among records in the three and four lactation groups where the number of daughter-dam pairs was small. This was evident among the 104 daughter-dam pairs having four or more records, where heritability estimates involving second records were −0.10, −0.07, and 2.35 for milk, fat, and percentage of fat, respectively. It is apparent that these estimates were subject to large sampling errors. Heritability estimates based on first records were lower among cows with two records than among cows with one, three, or four records. Similarly, estimates based on second records were higher than those based on first records among cows with two records. However, when cows with three or four records were considered.

first records yielded considerably higher heritabilities for milk and butterfat production than where second records were used.

The intrasire regressions and heritability estimates for 305-, 200-, and 100-day production for 646 first-record cows are shown in Table 3. The difference between the various regression coefficients for milk and fat are not significant. For percentage of fat, the difference between the 305- and 100-day regression is highly significant, whereas the 305- vs. 200- and 200- vs. 100-day regressions are not significant. These results are in general agreement with those of Madden *et al.* (20) and Rendel *et al.* (23), in that heritabilities based on 305-day milk and fat records are slightly higher than those based on lactations of shorter durations.

TABLE 3

Intrasire regressions (b) and heritabilities (h^2) of 100-day, 200-day, and 305-day production for 646 first-lactation daughter-dam pairs

Length of record	Milk		Butterfat		Test	
	b	h^2	b	h^2	b	h^2
100 days	0.151 ± .041	0.30	0.156 ± .043	0.31	0.222 ± .042	0.44
200 days	0.179 ± .041	0.36	0.158 ± .042	0.32	0.318 ± .039	0.63
305 days	0.211 ± .041	0.42	0.188 ± .043	0.37	0.402 ± .037	0.80

Repeatability estimates were determined, using the first four 305-day records of 440 cows sired by 87 bulls in 29 herds. These estimates were 0.47 for milk, 0.45 for fat, and 0.68 for percentage of fat, and are in general agreement with those reported by many workers using other breeds of dairy cattle. For milk yield the herd component comprised 25% of the total variance, sires 14%, and cows in sires 21%, leaving a residual component of 40%. The corresponding values for fat yield and percentage of fat were 24, 18, 17, 41 and 10, 8, 53, and 29%, respectively.

Heritability estimates obtained by doubling the intrasire regression of daughter on dam, and reported for various breeds of dairy cattle in the United States, are in the order of 0.20 to 0.40 for milk, 0.20 to 0.35 for fat, and 0.35 to 0.75 for percentage of fat. While the heritability estimates found in this study of Brown Swiss cattle are slightly higher, most of the estimates previously reported fall within the lower range of the fiducial limits reported here. It is doubtful if interherd variation had an important influence on these heritability estimates which were obtained on an intra-sire basis. Artificial breeding was not practiced in these herds and 85, 11, and 4% of the sires involved were represented in only one, two, and three herds, respectively.

It is concluded that under the environmental conditions prevailing in Brown Swiss herds on Herd Improvement Registry in Wisconsin, the effectiveness of selection and genetic improvement of production traits is similar to that in other dairy breeds. The moderately high repeatabilities (milk = 0.47, fat = 0.45, and percentage of fat = 0.68) and the heritabilities using first records (milk = 0.42, fat = 0.37, and percentage of fat = 0.80) indicate that culling can be effective after the first lactation. Mass selection based on partial first records of 100 or

200 days in length will be almost as effective in improving milk and fat yield as selection based on the complete 305-day first-lactation record. In the selection for percentage of fat, partial records will be considerably less effective than the complete 305-day first-lactation record.

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CRAMPY OR PROGRESSIVE POSTERIOR PARALYSIS IN MATURE CATTLE [1, 2]

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SUMMARY

Records from 12,387 dairy bulls of five breeds, and 448 beef bulls of four breeds, were studied to establish the frequency and age at occurrence of the crampy condition, and to determine if evidence of genetic influence existed. The 323 dairy and ten beef bulls which were affected represented all nine breeds. Chi-square analysis showed that the frequency of occurrence differed significantly ($P < 0.01$) among the dairy breeds. Death or last effective service of affected bulls occurred at a mean age of 10.02 ± 2.49 yr. Numerous familial relationships were demonstrated; differences in frequency of occurrence in sire groups, and in lines and line-crosses, were significant ($P < 0.04$ and $P < 0.01$, respectively). Thus, a genetic background of the condition was indicated strongly. Although multiple gene inheritance was not discounted, inheritance was suggested as a single recessive factor with incomplete penetrance.

A syndrome characterized by intermittent spastic contraction of the muscles of the rear legs and back of mature cattle of both sexes has been recognized for some years. It has been called crampy (krampfigkeit), neuromuscular spasticity, posterior paralysis, progressive posterior paralysis, spastic syndrome, stretches, and other names. The cause of this syndrome has not been established; however, a familial pattern of occurrence has been reported. Crampy has shortened the useful lives of numerous bulls and cows.

Crampy occurs in otherwise normal adult cattle. Symptoms become evident when animals are three or more years of age and usually are mild for the first few years. The earliest signs are slight muscle spasms which appear when animals arise, back up, or move suddenly. As the condition progresses the muscles of one or both rear legs go into spasmodic contractions that last for a few seconds to several minutes. Animals often extend or flex their legs, one at a time, during these episodes. When both rear legs are affected, they remain on the ground and are extended backward during an attack; hence, the term stretches. The condition becomes progressively more severe over a period of months or years until the muscles over the back, and eventually much of the body, are involved. Because physical activity is necessary to induce an attack, animals affected severely usually remain recumbent. Animals in the advanced stages have reduced value.

Roberts (4) observed or assembled reports of 65 cases, 33 bulls and 32 cows. Affected cattle included two full brothers, two half-brothers, three sons of the

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same bull, and a three-generation group of females. Five breeds were involved. Previously, Wirth and Diernhofer (6) stated that the syndrome might be determined by genetic factors.

Frauchiger and Hofmann (3) were unable to demonstrate histological changes in the central nervous system of mature cattle affected with krampfigkeit. Roberts (4) postulated that the condition resulted from a derangement of the postural reflex mechanism.

The objectives of this investigation were to establish the frequency of the condition in a population of bulls used in natural and artificial service, and to evaluate a possible genetic influence upon the occurrence.

EXPERIMENTAL PROCEDURE

A study of useful tenure and reasons for disposal of bulls from natural and artificial service has been under way for some years (1, 2). Records were accumulated mainly by direct correspondence with owners, managers, and herdsmen. Reasons for disposal were available for 12,387 dairy and 448 beef bulls, as well as the ages at death or last effective natural service or semen collection.

Five-generation pedigrees were compiled on affected members of the five dairy breeds, and familial relationships established. Standard statistical techniques (5) were applied where appropriate.

Animals were considered crampy if the reason for disposal was listed as crampy or one of its previously mentioned synonyms. Other conditions which might have been crampy, but which were excluded: unable to mount, unable to stand up on hind legs, and refused to serve. Paralysis alone was not considered crampy, since it could follow a stroke caused by an embolism in the brain; similarly, injury of a peripheral nerve that interfered suddenly with control of a part of the body was not considered. Some diagnoses of rheumatism and gradual loss of use of the hind quarters were accepted. Estimates of frequency of crampy would, thus, doubtless be somewhat conservative.

RESULTS

Frequency of occurrence. Some 323 dairy and ten beef bulls died or were removed because of crampy (Table 1). These bulls were removed from service at a mean age of 10.02 ± 2.49 yr., with a range of 3 to 16 yr. Affected bulls comprised 2.6% of the total population. Among the five dairy breeds, frequency ranged from 1.9 to 3.9%. Chi-square analysis showed that frequency of occurrence differed highly significantly ($P < 0.01$) among breeds in the records.

Pedigree study. Familial relationships were determined from the pedigrees of affected bulls. Many of the related individuals were in widely separated parts of the country, in natural or artificial service, and under what was assumed to be good or excellent management. From one to eight sons of certain bulls were affected; a number of dams had two sons affected, some in different herds.

TABLE 1

Age at death or disposal of crampy dairy bulls

	Ayrshire	Brown Swiss	Guernsey	Holstein-Friesian	Jersey
	Total removed [a]				
	1,232	803	3,166	4,145	3,041
Years	Number removed for crampy				
3	....	....	....	1	....
4	....	1	1	4	1
5	2	....	....	5	1
6	2	1	2	11	4
7	5	1	6	17	3
8	4	....	8	16	1
9	5	3	9	27	11
10	10	3	10	22	10
11	9	2	10	17	9
12	4	4	8	15	8
13	3	1	3	5	2
14	1	....	2	2	2
15	1	....	1	2	....
16	2	....	....	3	....
Total	48	16	60	147	52
Percentage	3.9	2.0	1.9	3.5	1.7

[a] Total number of dairy bulls over 3½ yr. at disposal was 12,387, with 323 removed or died when crampy.

One Ayrshire bull had seven crampy sons from a total of 29. The affected animals were 7 to nearly 16 yr. old at disposal or death. The remaining 22 sons were not reported as crampy at disposal, nor was the bull himself. Of the seven crampy sons, one was inbred, and another was out of the sire's paternal sister. Six of the seven sons traced through their dams to a common sire in the first, second, or third generation. Other affected descendants of this ancestor included four grandsons, a great-grandson, a double-great-grandson, a triple-great-grandson, and five great-great-grandsons. In another line, an Ayrshire bull had a crampy son, a crampy grandson, and the latter in turn had a crampy son. The last had a maternal brother affected likewise.

The 16 Brown Swiss bulls reported as crampy had the following pedigree relationships: (a) two were paternal brothers; (b) two were sired by full brothers; (c) a bull and his son were affected; (d) a bull and his grandson were affected, and (e) a bull and three-great-grandsons were afflicted.

A Guernsey bull left service at 10.6 yr. because of an injury to a stifle; his sire was removed at 9.1 yr. for stiff hind legs. Three sons of the first bull were discarded at 11.0 to 14.9 yr. for acute rheumatism, a twisted stifle and crampy, respectively. A grandson lost use of his hind legs at 12.4 yr.; another was slaughtered at 10.9 yr. for a back injury, and a third at 11.3 yr. for crampy. Two great-grandsons, themselves three-quarter brothers, were disposed of for crampy at 7.2 and 11.6 yr. Nine other more distantly related descendants tracing one to four times to the original bull were slaughtered at 7.7 to 11.1 yr. for crampy. In this line there were four successive generations of crampy bulls.

Among other Guernsey lines, there were two instances in which a bull, his grandsire, and two great-grandsires were crampy as well as a double-great-grand-

sire. These animals were removed at 6.3 to 13.5 yr. In another case, a bull, his sire, double-grandsire, and double-great-grandsire all were crampy.

Eight sons and nine grandsons of a popular Holstein bull were crampy. Another Holstein bull had eight sons and 14 grandsons that were crampy. Two of these affected sons were full brothers. Yet, other sons and grandsons left service for several different reasons other than crampy at 5 to 16 yr. Another outstanding bull lost the use of his left hind leg at 10.3 yr. His crampy descendants included a son, two grandsons, and four great-grandsons, of which two were full brothers and two others were three-quarter brothers. The dam of the two crampy full brothers was herself crampy. Another bull was himself removed for rheumatism in the hind legs at 8.6 yr.; one grandsire went out for paralysis in the rear quarters and the other for rheumatism. These were owners' statements.

In a different Holstein line, one bull was apparently normal, having left the herd because of old age at 14.7 yr. His sire had been removed at 14.0 yr. for an injury to a hind leg. The son, in turn, sired one affected bull, as well as having two affected grandsons and three great-grandsons. Two of the latter were fifteen-sixteenths brothers. One bull, his sire, and both grandsires went out of use because of the spastic condition, failure of the hind quarters, or paralysis, according to the statements of the respective owners. Two of these were full brothers. A bull, his son, and several of the daughters of each were crampy in an institutional dairy herd. A group of crampy Holsteins included a cow, her two sons, and a grandson. Her mates were closely related bulls; one died of unknown cause at 3 yr. and the other was reported to have an injured leg at 11.8 yr.

The following family relationships existed among afflicted Jersey bulls. An affected male had the following affected descendants: three sons, two grandsons, and six great-grandsons. One of the latter was a double-great-grandson. Another living great-grandson has been crampy since he was 4 yr. old. Three of the great-grandsons died in widely separated parts of the country, and the living crampy great-grandson; all were three-quarter brothers. In another line, a bull and son were affected; in another, a bull, grandson, and a great-grandson were crampy. Still another Jersey, which left a well-managed herd for a "mineral deficiency," had a grandson and two great-grandsons which were crampy at disposal when 6 to 12 yr. old.

Other breeds. Of 251 Milking Shorthorn bulls, crampy individuals included one each at 6, 8, and 9 yr. old. One crampy Angus bull out of 91 animals went out at 10 yr. old. A 6-yr. and an 11-yr.-old Beef Shorthorn were eliminated among the 32 of this breed. Two Herefords were crampy at 3 yr., and one each at 8 and 9 yr., among 74 animals. This was 2.2% of the 448 beef and dual-purpose animals.

Frequency in sire groups and lines. Pedigree study enabled the grouping of normal and affected bulls into sire groups (paternal half-sibs to full sibs). Where closely related bulls occurred, they were grouped into lines. Where members of a line were crossed with unrelated females, the sons were designated

TABLE 2

Frequency and distribution of crampy sons among sire-groups and among lines and line-crosses

Breed	Total No. of sons [a]	Number of crampy sons	Degrees of freedom	Chi-square
	Among sire-groups			
Holstein	183	26	20	26.9
Guernsey	183	10	25	36.0
Combined	366	36	45	62.8 [b]
	Among lines and line-crosses			
Holstein	235	35	18	33.1
Guernseys	257	17	22	33.8
Combined	492	52	40	66.9 [c]

[a] In groups of four or more.
[b] $P < 0.04$.
[c] $P < 0.01$.

as a line-cross. The requirement was added that a sire group, or a line or line-cross, must have consisted of at least four individuals. Only Guernsey and Holstein bulls past 5 yr. old were included in the chi-square analysis.

As shown in Table 2, 366 males representing 47 sire groups and 492 males of 42 lines and line-crosses were utilized. It was assumed that if crampy were due to nongenetic factors it would occur in these categories in a random fashion. Chi-square analysis showed that this hypothesis was rejected at the 4 and 1% levels, respectively. Besides the frequencies of affected animals mentioned previously, additional groups were relatively free of the condition. Among sire groups the following extremes were noted: zero of 18 sons and zero of 19 sons; among line-crosses: zero of 19 and one of 61 sons.

DISCUSSION

Evidence of genetic influence on crampy appears almost conclusive. Frequencies of the condition in the five dairy breeds differed significantly; crampy occurred in cattle within closely bred family lines and did not occur in other closely bred but unrelated lines. The character developed in animals where both sire and dam were affected. Familial relationships, although not convincing individually, seem too numerous to be discounted.

Perhaps the exact mode of inheritance may never be learned. It is entirely possible that multiple gene inheritance may be involved, with the condition inherited in the manner of many quantitative characteristics. However, the condition may be inherited as a qualitative characteristic. There were numerous instances from pedigree analysis that followed the pattern of that of recessive red hair-color. The manifestation of crampy may be attributable to incomplete penetrance.

The condition is neither sex-limited nor sex-linked. It occurred in bulls and cows, as well as in first-generation progeny of affected animals of either sex. It is not dominant in character, since it frequently skipped generations in expressing itself. If it were assumed that crampy was inherited as a simple recessive, then its gene frequency would be about 16.2%, with 2.6% of the geno-

type homozygous recessive (crampy), 27.1% heterozygous (or carriers), and 70.3% homozygous dominant, or free from the gene. Incomplete penetrance would necessarily make this estimate of gene frequency low, since many animals doubtless left the herds before the condition was recognized, and thus were assumed free. These suppositions do not preclude the possibility that polygenic inheritance may be involved.

The average age at disposal (10.02 yr.) was higher than the over-all age of disposal of the desirable bulls from artificial service (1, 2). Affected animals were discarded as young as 3 yr., however, and some bulls transmitting high milk production would have been kept longer, had it been possible.

In the past, some crampy bulls have been used in the advanced stages, being treated with pain-relieving drugs and collected sometimes with electro-ejaculators when unable to mount. This indicated esteem of these individuals because of desirable characteristics. The popularity of their respective progeny indicated regard for their ability to transmit milk and butterfat production. The crampy condition is distributed widely enough and is of sufficient importance in shortening the useful lives of some dairy animals that some consideration should be given when breeding to known affected bulls. Breeders may wish to consider crampy when selecting future herd sires, should their ancestry be known as carriers.

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LEVELS OF DEOXYRIBONUCLEIC ACID IN BOVINE SPERMATOZOA AND THEIR RELATIONSHIP TO FERTILITY [1, 2]

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SUMMARY

The chemical determination of DNA in 71 ejaculates of 17 bulls indicated that on the average, one billion spermatozoa contain about 2.95 ± 0.17 mg. of DNA. Data on 34 ejaculates of ten bulls revealed an over-all correlation of 0.20 (not significant) between DNA level and fertility. On an among-bull basis, the correlation was -0.35 (not significant) and among ejaculates, within bulls, it was 0.43 ($P < 0.05$). A study of ten ejaculates from two bulls indicated a nonsignificant decline of 3% of DNA content during storage of raw semen at room temperature. No decline was observed at 5° C. Data from 20 ejaculates indicated that both high and low levels of DNA were associated with increased percentages of delayed returns to heat after service. Uterine and oviduct luminal fluids of slaughtered cows contained 4-6 mg. of DNA per milliliter. This is probably derived from epithelial cell nuclei.

Many semen evaluation techniques based on concentration of spermatozoa, motility, morphology, and various measures of metabolism have been tested and found to have rather limited value in predicting fertility of relatively normal semen (10). The recent work by Leuchtenberger (8) regarding the relation of DNA (deoxyribonucleic acid) in sperm cells to fertility in men and bulls is of interest. She reported that spermatozoa of infertile males showed marked variations in DNA content with average levels being significantly lower than for fertile males. The low and high levels appeared to originate in the spermatogonia, since the ratio of 4:2:1 for DNA levels in primary spermatocytes, secondary spermatocytes, and spermatids remained the same even when gross amounts were abnormal.

Cells undergoing growth and mitosis undoubtedly possess the necessary substrates and enzyme systems for the synthesis of DNA. In nongrowing cells, the metabolic turnover of DNA is presumably very slow (5). Since mature spermatozoa are apparently incapable of growth and mitosis, it seems likely that the prerequisites for DNA synthesis are lacking (perhaps the migration of mitochondria to the mid-piece area removes the source of oxidative chemical energy from the sperm head proper). It would also seem likely that any net degradation of sperm DNA would result in mutation-like changes, some of which might be lethal to resulting embryos. The cooling of semen might be an important factor in reducing the already slow rate of DNA degradation. Salis-

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bury *et al.* (11) have reported that the proportion of delayed returns increased as age of the semen increased. This would seem to indicate that embryonic mortality increases with aging of semen. Salisbury *et al.* (12) also reported a marked decrease in the apparent DNA content of spermatozoa during storage for two to ten days in yolk-citrate at 5° C. In addition, several reports by Blandau *et al.* (2, 3, 13) have indicated that embryonic mortality increases with aging of eggs prior to fertilization.

The following study was undertaken for the purpose of determining (a) if fertility of sperm is related to their DNA content, (b) if there is a decline in the DNA content of sperm during storage, and (c) if DNA level of sperm has any relation to embryonic mortality as reflected in the proportion of delayed returns to service.

EXPERIMENTAL PROCEDURE

The procedure for determination of DNA was a slight modification of the Schmidt-Thannhauser method (6). One-milliliter aliquots of semen of known spermatozoan concentration were mixed with 10 ml. of a 3% sodium hydroxide solution and incubated at 37° C. for 24 hr. Then a 5-ml. aliquot was treated with 1 ml. of 6 *N* HCl and 5 ml. of 5% trichloracetic acid. The mixture was then centrifuged for 5 min. The supernatant liquid was discarded and the residue was resuspended in 5 ml. of 5% trichloracetic acid. This mixture was then heated to 90° C. in a water bath for 15 min. After cooling, the mixture was centrifuged and the supernatant retained. The residue (protein) was washed with 2.5 ml. of 5% TCA, centrifuged, and the supernatant retained. The combined supernatants contained the DNA, which was estimated by determining deoxyribose according to the method of Dische (4), or by determining phosphate according to the method of Fiske and Subbarow (7). For purposes of calculation, DNA was considered to be 40% deoxyribose and 9.89% phosphorus.

RESULTS

Determinations of DNA were made on aliquots of 71 ejaculates from 17 bulls which were being routinely used for artificial insemination service. The average content of DNA was found to be 2.95 ± 0.17 mg. per billion sperm, with a range of 1.77 to 4.77 mg. This level is somewhat lower than the 3.3 mg. per billion sperm reported by Bhargava *et al.* (1) and Vendrely and Vendrely (14). Fertility data were available for 34 ejaculates of ten bulls in which each ejaculate was used to breed 30 or more cows (average 67). The over-all correlation coefficient between fertility and DNA level was 0.20 (not significant), whereas that among ejaculates within bulls was 0.43 ($P < 0.05$). On the other hand, the correlation coefficient between fertility and DNA level on an among bull basis was −0.35 (not significant). Thus, it appears that certain bulls may have a detrimental excess of DNA in their sperm cells. However, within the levels characteristic of bulls, it appears that certain ejaculates may show a detrimental decline in DNA content. Whether this is associated with losses of DNA during maturation or aging of sperm in the male organs could not be determined from this study.

Ten ejaculates from two bulls were used to determine whether there is a detectable decline in the DNA content of spermatozoa during storage of the raw semen (with antibiotics added) for 72 hr. at either room temperature or at 5° C. There was a statistically nonsignificant decline of 3% in DNA content at room temperature, but no decline during storage at 5° C.

Assuming that the decline in fertility between 30-60 and 60-90 day nonreturns is proportional to embryonic mortality, data from 20 ejaculates, each used to breed 30 or more cows (average 72), indicated that both high and low levels of DNA might tend to increase embryonic mortality. The average decline in percentage nonreturns between 30-60 and 60-90 days was about five to eight percentage units for three ejaculates containing less than 2.25 mg. DNA per billion sperm and for 11 ejaculates containing 3.00 mg. or more of DNA per billion sperm. However, for five ejaculates containing 2.25 to 2.99 mg., the average decline in nonreturns was only 2.1 percentage units.

DISCUSSION

While the present data are somewhat restricted in volume and many of the trends were not statistically significant, the indications are certainly interesting and the problem is deserving of further study. As a sidelight of the present study, determinations of DNA were made on five samples of uterine and oviduct luminal fluids from slaughtered cows. It was found that these fluids contained 4-6 mg. of DNA per milliliter, which is about twice the concentration found in semen. This was not surprising in view of the large number of epithelial cell nuclei reported in these fluids by Olds and VanDemark (9). It would seem likely that this DNA (or its breakdown products) might be utilized by cleaving ova and developing embryos, since each mitotic division requires a preliminary doubling of the DNA content.

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TECHNICAL NOTES

PHOTOMICROGRAPHS OF MILK PROTEIN PARTICLES

Sedimentation studies have shown that some of the protein particles in untreated raw milks should be microscopically visible (1, 4). When milks were heated to sterilization temperatures, the number of larger particles increased. Sedimentation constants of as high as $38{,}000 \times 10^{-13}$ were obtained for concentrated skimmilks which were reconstituted to their normal solids content immediately after sterilization (3). The diameter of the protein particles was calculated by the equation $d = \sqrt{\frac{18\,\eta\,s}{\rho - \rho_o}}$ in which d is diameter in cm., η, viscosity in poises, s, the sedimentation constant, and $\rho - \rho_o$ the densities of the particle and solution, respectively. The diameter was found to be 0.7 μ. Microscopic examination confirmed the calculated size. The size most numerous in sterile concentrated whole milks had a diameter of about 2 μ. Under a phase microscope, the particles appeared to be fat globules with adsorbed proteins, and the calculated difference in density between the particles and the solution was about 0.1.

To study further the changes in sizes of protein particles, photomicrographs were made. A 2% agar solution was placed on a microscope slide and allowed to gel (2). About 0.001 ml. of milk was placed on the agar film, covered with a cover slip, and immediately placed under the oil immersion objective of a phase microscope. Satisfactory pictures were obtained with 1-sec. exposures on high-contrast copy film. Exposures of 1/125 sec. on Tri-x film also yielded satisfactory pictures, but the shortest exposure possible did not stop the Brownian movement of individual particles when the milks were placed under cover slips on microscope slides without the agar film.

Pictures representative of the results obtained are shown in Figure 1. Picture A was undiluted sterile whole milk which had been stored at 70° F. for 30 days. The particles were similar to, but smaller, than those found in sterile concentrated whole milks. Picture B shows the stage micrometer at the same magnification as all of the pictures in Figure 1. The distance between the centers of the marks is 10 μ. It appears that only one of the particles of the whole milk was as large as those most numerous in sterile concentrated whole milk. The halo around the particles was typical of fat particles under the phase microscope. Pictures C, D, E, F, G, and H were, respectively, centrifugally separated raw skimmilk, the same milk after preheating at 180° F. for 4.16 sec., reconstituted immediately after vacuum concentration, reconstituted after the concentrated milk had been sterilized at 290° F. for 2.08 sec., reconstituted after storage for 14 days at 100° F., and sediment that settled during storage at 100° F. Brownian movement was not stopped completely and the smaller particles visible in pictures C, D, and E blurred the pictures.

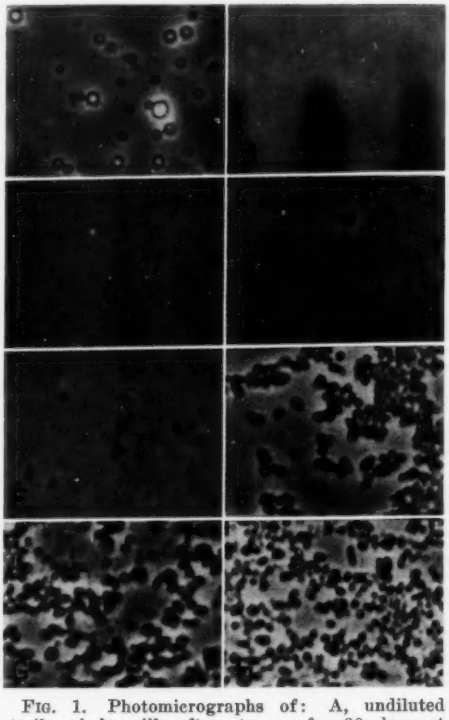

FIG. 1. Photomicrographs of: A, undiluted sterile whole milk after storage for 30 days at 70° F.; B, stage micrometer at same magnification; C, raw skimmilk; D, skimmilk heated to 180° F. for 4.16 sec.; E, reconstituted after vacuum concentration; F, reconstituted after concentrated skim was sterilized at 290° F. for 2.08 sec.; G, reconstituted after storage at 100° F. for 14 days; H, sediment during storage 100° F. for 14 days.

From pictures (Figure 1) C, D, and E it is evident that there was no great difference in the size of particles as a result of heating to 180° F. for 4.16 sec. These small differences correspond to the typical sedimentation curves for raw milk and the same milk after it had been heated to 180° F. (Figure 2). A great change in size occurred when the concentrated milk was heated to 290° F. for 2 sec. This change in size corresponds to an increase in the sedimentation constant from approximately 400×10^{-13} for raw milk (Figure 2) to $38{,}000 \times 10^{-13}$ for reconstituted sterile concentrated skimmilk (Figure 3). The increase in the number of smaller particles (Figure 1, G) is shown in the sedimentation curve of the same milk reconstituted after storage for 13

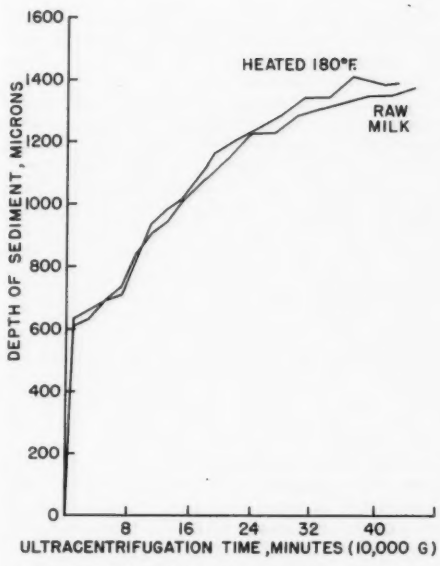

FIG. 2. Sedimentation curves of raw milk and same milk heated 180° F. for 4.16 sec.

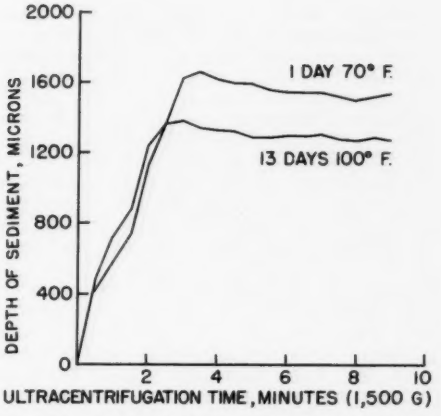

FIG. 3. Sedimentation curves of reconstituted skimmilk in Figure 1, F and G.

days at 100° F. (Figure 3). A difference not shown clearly in the pictures is that immediately after processing (Figure 1, F) the individual protein particles showed Brownian movement until it was stopped on the agar film, whereas the milk reconstituted after storage (Figure 1, G) had numerous groups and short chains with slower Brownian movement.

A picture of the sediment (Figure 1, H) which settled during storage at 100° F. for 14 days did not show larger particles than those that remained in suspension in the milk.

This photomicrographic technique can be useful in further studies of protein particle size and aggregation in fluid milk products.

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BLOAT IN CATTLE. II. ITS DEVELOPMENT ON ALFALFA PASTURE AFTER INOCULATION OF EMPTY RUMINA WITH AUTOCLAVED OR FRESH RUMEN FLUID [1]

Recent research has indicated a relationship between rumen microflora and bloat. It has been suggested that slime production by rumen microorganisms contributes to feed-lot bloat (7) and to frothy legume bloat (6). Bacteria capable of degrading alfalfa saponin with a concomitant production of slime have been isolated from the rumen (4). An increase in the numbers of *Streptococcus bovis* type streptococci and *Peptostreptococcus elsdenii* during the onset of feed-lot bloat has been observed (5). Temporary control of bloat by antibiotics (1, 2) further suggests an association between microorganisms and bloat. This investigation was undertaken to determine whether an animal would bloat on legume pasture after being deprived of practically all its rumen microflora by completely emptying the rumen before pasturing.

Four sets of fistulated identical-twin dry

[1] Contribution No. 293, Department of Dairy Husbandry; No. 365, Department of Bacteriology, Kansas Agricultural Experiment Station, Manhattan, Kansas.

cows were pastured in early June on a succulent second-growth alfalfa pasture that provoked severe frothy bloat in a majority of other animals. One set of twins (A1-A2) was pastured daily and served as a control. On the initial day of the trial at 6 A.M. the three other sets (B1-B2, C1-C2, D1-D2) had their reticulorumina completely emptied and washed with water till the washings were clear. Four liters of freshly collected and strained rumen fluid obtained from the control pair (A1-A2) was introduced through the fistula into the rumen of one member of each twin pair (B1, C2, D1—selected at random). Four liters of rumen fluid collected from A1 and A2 was strained and autoclaved and then introduced into the rumen of the other member of each twin pair (B2, C1, D2). The latter rumen fluid was collected the previous day and autoclaved for 20 min. at 121° C. After treatment, the animals were staked in pasture so that the quantity of feed consumed could be estimated. All four sets of twins were observed for degree of frothing of rumen contents at 2-hr. intervals during the day (1-hr. intervals during the first 2 hr.). They were placed in dry-lot at 3 P.M. each day and returned to pasture at 8 A.M. the next day. The duration of the first experiment was six days. The experiment was repeated with the treatments reversed.

The degree of frothing was scored, using a 0-5 scale. A 5 maximum was given when frothy rumen ingesta gushed out several feet when the cap closing the fistula was removed.

Rumen samples were obtained for bacteriological analyses approximately 4 hr. after the cows were turned into the pasture. The following examinations were made: (1) a direct microscopic observation of gram-stained slides of rumen fluid diluted 1 to 10, (2) plate counts of aerobes able to grow on tryptone-glucose-yeast agar, (3) percentage of flora resembling lactic acid–producing types as characterized by reaction in litmus milk, and (4) cellulolytic activity of rumen contents using a test tube method (3).

In both experiments all the animals consumed alfalfa pasture the first day. However, the feed consumption by animals inoculated with autoclaved rumen fluid was noticeably less than by those inoculated with fresh fluid. The latter group consumed slightly less feed than the control pair. By the third day no observable difference in feed consumption existed among groups. Since feed was not weighed, these observations are empirical.

Data in Table 1 show that the pasture was bloat-provoking, since the control cows (A1-A2) reached their maximum ability to froth during most days in both Experiments I and II. Under these conditions the cows inoculated with fresh rumen fluid in Experiment I did not produce froth the first day, but by the second day reached their maximum ability to froth. The cows inoculated with the autoclaved fluid did not reach maximum ability to froth until the fourth day. When the treatments were reversed (Experiment II), the results of Experiment I were almost duplicated, with severe frothing occurring on the second day in cows inoculated with fresh fluid and no marked degree of frothing occurring in the group inoculated with autoclaved fluid until the fourth day. Inocula obtained from cows 4 hr. after adding autoclaved fluid digested filter paper more slowly than inocula from cows receiving nonautoclaved fluid. This difference generally disappeared after one day. Also, cellulolytic activity of rumen fluid from both groups of inoculated cows was less than that of the controls during the first day of the experiments, but after two days it almost equalled that of the controls. Inocula from bloated animals digested filter paper more slowly than did inocula from nonbloated animals.

At the start of each experiment rumen samples from animals receiving autoclaved fluid had higher aerobic counts than those receiving nonautoclaved fluid. With the appearance of bloat on the third day (in animals receiving autoclaved fluid) aerobic counts diminished. Throughout Experiment I there was no marked difference in the percentage of lactic-type organisms in rumen fluid between cows receiving autoclaved and those receiving nonautoclaved fluid. Throughout Experiment II the percentage of lactic-types was higher in the rumen fluid of animals receiving autoclaved fluid. Direct microscopic examination of gram-stained slides of rumen fluid showed no consistent differences in numbers or types among groups, although there were variations between samples. The original autoclaved rumen fluid had approximately the same number and morphological types of stained bacterial cells as fresh rumen fluid. Presumably, the dead organisms, at least in early samples, complicated efforts to evaluate the flora microscopically. However, as the experiments progressed the organisms tended to be present in clumps and masses. This condition seemed to be associated with the more viscous rumen samples and suggested the presence of a gelatinous-like material. However, it was not particularly correlated with bloat. It was more difficult to obtain good staining of such preparations than of those from less viscous samples showing less clumping of bacteria. It was later noticed that this clumping was much less when cows consumed alfalfa hay rather than alfalfa pasture.

Results of the present study may be interpreted as follows: (1) Cows deprived of practically all their rumen microflora can consume normal quantities of alfalfa pasture by the third day and (2) can bloat by the fourth day. It might be conjectured that an animal devoid of rumen flora develops a functional flora from its feed in a few days. These results also sug-

TABLE 1

Effect on frothy bloat of inoculation of empty rumina of identical twin cows with either autoclaved or fresh rumen fluid [a]

	Maximum degree of frothing [b]										
	Autoclaved fluid				Fresh fluid				Control		
	Cow No.				Cow No.				Cow No.		
Date	B2	C1	D2	Av.	B1	C2	D1	Av.	A1	A2	Av.
	Experiment I										
6-3	0	0	0	0	0	0	0	0	5	5	5.0
6-4	0	0	2	0.7	2	4	5	3.7	5	5	5.0
6-5	2	0	2	1.3	2	3	3	2.7	5	5	5.0
6-6	5	2	5	4.0	0	5	2	2.3	5	3	4.0
6-7	4	4	2	3.3	0	4	0	1.3	3	0	1.5
6-8	3	2	5	3.3	3	5	0	2.7	5	5	5.0
	Experiment II										
	Cow No.				Cow No.				Cow No.		
	B1	C2	D1	Av.	B2	C1	D2	Av.	A1	A2	Av.
6-9	0	0	0	0	0	0	0	0	5	5	5.0
6-10	0	0	0	0	5	5	5	5.0	3	3	3.0
6-11	1	1	0	0.7	5	5	5	5.0	5	5	5.0
6-12	4	2	3	3.0	5	5	5	5.0	5	5	5.0
6-13	5	2	5	4.0	5	3	2	3.3	2	2	2.0
6-14	5	5	5	5.0	4	3	4	3.7	4	3	3.5

[a] Four liters of strained autoclaved or strained fresh rumen fluid on first day of each experiment.

[b] Maximum degree of frothing observed during the day, based on rating from 0 (no froth) to 5 (severe froth).

gest that microflora are involved in bloat and that bloat is not the result of simple physical breakdown of feed, since the cows did not reach maximum bloat proportions during the second or third day, even though consuming almost optimum amounts of bloat-producing feed. Further association of the rumen microflora and bloat is suggested by reactions of the group of cows receiving autoclaved fluid differing from those receiving fresh fluid. The cows in the latter group bloated sooner, perhaps because bloat depends on establishing a certain concentration of microorganisms. The reduced cellulolytic activity of the rumen fluid plus higher aerobic counts during the first day in the group receiving autoclaved fluid supports such a hypothesis.

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CONVERSION OF CAROTENE FROM ALFALFA AND FROM WATER-DISPERSIBLE GELATIN BEADLETS TO VITAMIN A BY CALVES [1]

In conducting studies dealing with vitamin A nutrition of calves, it is desirable to have a source of the provitamin, carotene, which is not only concentrated and stable but also relatively free of other nutrients. The recent development (2) of a dry stabilized form in which carotene is incorporated into water-dispersible gelatin beadlets appeared to meet these requirements. Therefore, the present study was undertaken to determine the conversion to vitamin A of this source of carotene and to compare it with that from alfalfa. Alfalfa was used as a standard of comparison, since to date it has been employed in many of the vitamin A nutrition experiments undertaken at this Station.

Four Guernsey and 12 Holstein one-day-old male calves were obtained during March-May, 1959, from various Connecticut state institution herds, placed in individual sawdust-bedded tie-stalls, and raised to 63 days of age essentially as previously described (4). On the 64th day of age, each calf was fed a vitamin A depletion ration (4) at a level of intake to provide an anticipated seven-day increase in live weight of 10 lb. When the blood plasma vitamin A concentration had decreased to equal to or less than 12 γ per 100 ml., each calf (average age was 73 $\pm$ 4 days) was then fed after a seven-day period, one of two carotene intakes, 48 or 240 γ per pound of live weight per day, from one of two sources, artificially dehydrated alfalfa leaf meal or water-dispersible gelatin beadlets (Hoffmann-La Roche dry β-carotene beadlets, Lots No. 026 and 035, guaranteed to contain 2.4% β-carotene, dissolved in vegetable oil and dispersed in a gelatin-sugar-starch matrix with added food grade antioxidants). The first four calves of a breed to arrive at the research barn were assigned to a particular level of carotene intake and source according to a previous random allotment. This procedure was repeated until four blocks (one Guernsey and three Holstein) of four calves each were obtained. After 12 wk. of carotene supplementation (comparison period), calves were slaughtered.

Other procedures of this experiment, as well as observations and analyses, were essentially the same as those of a previous study (4). An exception was the feeding, first in the morning, of the carotene supplement made up to 1.0 lb. with approximately 600 ml. of water, followed by feeding the remainder of the vitamin A depletion ration allowance after each calf had consumed the initial amount. Average minimum and maximum temperatures during the carotene supplementation period were, respectively, 65.3 $\pm$ 0.4° F. and 77.5 $\pm$ 0.4° F. Average light intensity during this same period with incandescent light, restricted to 12 hr. daily, was 3.2 $\pm$ 0.7 foot-candles. Average carotene concentrations of alfalfa (1, 4) and beadlets (2, 5) were, respectively, 190.7 $\pm$ 2.1 γ per gram and 25,493 $\pm$ 153 γ per gram. The chemical composition of the feeds fed was essentially as previously reported (4) and, therefore, will not be included here. The analysis of variance of these data followed that for a randomized-block two-dose factorial assay. Since in a previous study (4) an interaction in ability to convert carotene to vitamin A between Guernsey and Holstein calves was not demonstrated, breed was not included as a separate source of variation. One calf, a Guernsey, fed the 48-γ carotene intake from alfalfa exhibited scours unaccompanied by elevated rectal temperature for approximately 10% of the carotene supplementation period and had quite low concentrations of carotenoids and vitamin A in the plasma and liver. Since scours decrease the intestinal absorption of preformed vitamin A (8) and, presumably, carotene, this calf's data were rejected and missing values calculated according to procedures for a randomized block design.

During the carotene supplementation period, calves readily consumed their vitamin A depletion ration allowance plus carotene supplement. The average percentage days free of ration refusals was 99.8 (the average arcsin $\sqrt{\text{percentage}}$ equaled 89.6 with a standard deviation of 3.3). With the exception of the one calf cited above, scours was not a problem in this experiment, the average percentage days free of scours being 99.6 (the average arcsin $\sqrt{\text{percentage}}$ equaled 88.8 with a standard deviation of 3.5). The average live weight the day prior to carotene supplementation was 162 lb. During carotene supplementation, the average daily gain was 1.63 lb., with a standard deviation per calf of 0.18. Upon statistical analysis, this growth criterion was found not to be affected either by source or by intake of carotene.

Plasma and liver carotenoids and vitamin A concentrations are presented in Table 1. Calves fed alfalfa had consistently greater average concentrations of carotenoids and lower average

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TABLE 1

Conversion of carotene from artificially dehydrated alfalfa leaf meal (alfalfa) and from water-dispersible gelatin beadlets (beadlets) to vitamin A by calves

	Plasma					Liver			
	Carotenoids		vitamin A			Carotenoids		vitamin A	
	Initial	Comparison period—av.	Initial	Comparison period—av.	Adjusted[e] comparison period—av.	Actual	Log	Actual	Log
Carotene intake[a]	——(γ/100 ml.)——					——(γ/100 g. wet tissue)——			
Alfalfa									
48 γ[b]	17	33	7.3	10.2	10.3	47[f]	1.73	173[f]	2.13
240 γ	22	90	7.0	18.8	19.3	158	2.19	1,225	3.03
Beadlets									
48 γ	12	24	7.4	12.0	12.0	50	1.67	213	2.29
240 γ	18	72	7.8	22.7	22.1	124	2.02	1,663	3.21
Standard deviation per calf	4	8	1.6	2.7	1.5	....	0.07	....	0.22
Rate of linear change per log γ carotene intake									
Alfalfa	....	+83 ± 8[c]	....	+12.3 ± 2.7	+12.9 ± 1.5	....	+0.65 ±0.07	....	+1.28 ±0.23
Beadlets	....	+70 ± 8	....	+15.3 ± 2.7	+14.4 ± 1.5	....	+0.56 ±0.07	....	+1.31 ±0.23
Sources combined	....	+76 ± 6	....	+13.8 ± 1.9	+13.7 ± 1.1	....	+0.61 ±0.05	....	+1.30 ±0.16
Relative value of beadlets to alfalfa	0.66		1.60		1.48	0.70		1.35	
	0.48–0.88[d]		0.95–3.02		1.06–2.22	0.51–0.94		0.85–2.26	

[a] Per pound of live weight per day.

[b] In this carotene intake group, one calf's data were rejected because of a high incidence of scours and its respective values calculated according to procedures for a randomized block design, except as indicated in [f] below.

[c] Standard error of the linear rate of change, s_b.

[d] 95% confidence limits.

[e] Values adjusted by covariance for initial values obtained the day prior to carotene supplementation.

[f] Average for three calves.

concentrations of vitamin A than did calves fed the water-dispersible gelatin beadlets. In the last row of Table 1, the relative values of with vitamin A depletion ration and mixed the beadlets to alfalfa, as determined by parallel line bioassay procedures, are given. Based on these data, the carotene from the beadlets was converted to vitamin A 1.42 times (unweighted average of the ratios in Columns 5 and 9 of Table 1) as effectively as the carotene from alfalfa.

The more efficient conversion to vitamin A of the beadlets appears to agree with those data of most previous investigators, as reviewed by Deuel (3) and Moore (7), in that carotene in oil has generally been reported to be a superior source of provitamin A activity than vegetable sources, and aqueous dispersions or colloidal suspensions of carotene were more efficient than carotene in oil. However, exceptions can be found in the literature, such as the study of King *et al.* (6), who found carotene in alfalfa more efficiently converted to vitamin A than carotene in oil. The greater concentration of carotenoids in the plasma and liver of the calves fed alfalfa than those of calves fed the beadlets was of interest. The determination by chromatography of the structural forms of carotene and other carotenoid pigments present in the supplement fed, particularly the alfalfa, and the plasma and liver, are needed to ascertain the biological significance of these observations. In the meantime, the water-dispersible gelatin beadlets appear to be a useful source of β-carotene for employment in vitamin A nutritional studies, as well

as those which deal with interrelationships between carotene and other fat-soluble vitamins.

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OUR INDUSTRY TODAY

TRENDS IN ULTRA-HIGH TEMPERATURE PASTEURIZATION[1, 2]

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During recent years, there has been a tendency to increase the temperature of pasteurization or heat treatment of many dairy products. Pasteurization was begun initially to make milk safe, and later the benefits of improved shelf-life became apparent. The standards for pasteurization were controlled within narrow limits because of the adverse effects on creamline and oftentimes on flavor. However, with the development and rapid acceptance of homogenized milk, it was no longer necessary to control the pasteurization treatment so closely. As a result, dairy plant operators began to increase the temperature and time of pasteurization for homogenized milk. They found that there was an improvement in shelf-life, and that this in turn permitted handling and distribution practices that have resulted in tremendous changes in the dairy industry. Some of these changes are concentration of plant facilities, less frequent deliveries, and extension of sales areas.

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At this point, one might ask—What is the goal of the industry in regard to shelf-life? Perhaps the ultimate in fluid milk is a sterilized product that will have a shelf-life of 3 to 6 mo. without refrigeration and a fresh fluid milk that will keep up to 30 days with refrigeration. Both of these goals are available to the fluid milk industry today. Some aspects of processing and packaging have not been worked out on a practical basis, but the goals are attainable.

In addition to the benefits of safety and extended shelf-life, the dairy industry has used heat treatments to develop certain desirable characteristics in its products. The ice cream processor has believed for many years that his product was improved when the milk proteins were altered by heat. Buttermilk and other products are given a specific heat treatment, not only for bacterial destruction, but also because certain body and texture characteristics are required.

Burton (2) probably summarized the potentials of UHT (ultra high temperature) best when he pointed out that in the effective range of temperatures, resistant spores in milk are destroyed about 30 times more rapidly for every 10° C. (18° F.) rise in treatment temperature. Chemical changes such as color development take place about three times more rapidly for every 10° C. (18° F.) rise in temperature. This means that a higher temperature treatment produces a greater bacteriological effect for a smaller chemical change. He uses the example that a treatment of 230° F. for 30 min. is equivalent bacteriologically to 248° F. for 1 min. However, the chemical effects will be ten times less.

These factors, along with others, explain why dairy plant processors are interested in UHT heat treatments of dairy products.

What is ultra-high temperature pasteurization? Industry practice indicates that the temperature range begins between 190 and 210° F. and may extend to 300° F. or above. The time is less well defined, but may be anything less than 1 sec. up to many seconds.

Another term, practical or commercial sterilization, should be mentioned. It refers to that condition whereby all of the bacterial spores may not be destroyed, but those that remain are inactive or will not grow under the ordinary temperatures of storage. Commercial sterilization is usually accomplished at 285° F. for 15 sec. to 300° F. for 0.5 sec.

REGULATORY PROBLEMS

Obviously, these treatments are far in excess of those essential for assuring the safety of the finished products. This fact has been recognized by the U. S. Public Health Service (18), but general agreement has not been reached by all concerned as to the minimum temperature-time relationships that should be established to insure adequate pasteurization.

Time does not permit an extended elaboration of the problem, but a little more discussion may help you to be aware of its magnitude. Since specific regulations have not been developed for much of the UHT equipment, some dairy plants must pasteurize by accepted

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[2] Presented to the Manufacturing Extension Section at the 55th Annual Meeting of the American Dairy Science Association, June, 1960.

methods and then use UHT treatments in addition. This results in excessive equipment and controls. However, before standards can be established, agreement must be reached as to the basic objective(s) of the pasteurization requirement. Then, the best scientific data can be used to set minimum standards. For example, pasteurization at 143° F. for 30 min. was established on the basis of scientific evidence for the thermal destruction of pathogenic bacteria, plus as great a margin of safety as possible without damaging the creamline of milk. This standard is applied to high fat products such as cream and sugar-containing products, such as chocolate milk. The standard for ice cream mix was set at 155° F. for 30 min. because there was evidence to show that fat, sugar, and total solids levels affected bacterial destruction. While it was recognized that this requirement was higher than it needed to be, it would work no hardship on the ice cream industry. There is no problem as long as the higher temperature–shorter time treatments are computed on the equivalence of these basic standards. However, as the industry has moved toward the UHT range, there has been a tendency to equilibrate the requirements with the more rigid standard of 155° F. for 30 min. For example, a direct steam injection system was approved as a process of pasteurization at 194° F. for both milk and ice cream mix. A heat exchange system was approved at 200° F. with a calculated hold of 3 sec. Both of these are equivalent to or higher than 155° F. for 30 min.

Some problems that arise if the higher standard is applied as minimum are (1) a loss of flexibility of use of equipment for certain products such as pasteurization of milk for making cottage cheese, (2) possible loss of efficiency of heat transfer on certain types of equipment operated for extended periods of time, (3) probable damage to the quality of some products, and (4) increased operating cost because of heating to higher temperatures.

It would seem to me that general agreement must be reached on the minimum requirements for pasteurization with adequate but not excessive safety margins. Then, the equipment or processes should be tested for compliance with these standards. Obviously, the manufacturer would have to prove that every particle of product received minimum treatment, the process had adequate controls and the design, construction, and material features were satisfactory. Then it could be left primarily to the dairy plant operator and equipment manufacturer to adapt the equipment to his particular needs beyond the minimum requirements.

ULTRA-HIGH TEMPERATURE EQUIPMENT

There are several types of equipment whereby UHT processing can be accomplished.

Herreid and associates (11, 12) at Illinois demonstrated that a heat exchanger, a machine with tubes of small diameter through which the milk moved at high velocities, could be used for UHT pasteurization. The flavor score of the milk when heated to 300° F. was 39. Most commercial milk scores between 38 and 40 on flavor, so this treatment could not be considered deleterious to the milk. Presumably, milk could be processed at lower temperatures in this equipment with excellent results.

Another heat exchange unit (7, 16), consisting of a hollow stainless steel core with a spiral giving it the resemblance of an auger, is being used in milk and ice cream processing. This spiral provides a small annular space between the core and the cylinder, through which a thin film of product moves at high velocity. Heat supplied by steam is applied to both sides of the flow pass. This process has been approved for pasteurization at 200° F. for a calculated hold of 3 sec. Bacteria counts are reported to be below 500 per milliliter and often much lower. The equipment can be operated at higher temperatures with longer holding times. The flavor of the finished products is reported to be excellent, with a shelf-life under normal refrigeration of 30 days or longer.

Another heat exchanger (9) composed of two elements: "An outer jacketed shell, and an inner, slightly cone-shaped element with helical grooves having a cross section of approximately 0.01 sq. in." is used for sterilizing milk. Product moves at 23-24 ft/sec in turbulent flow. Temperatures are 285° F. or higher. The milk is deaerated prior to the heat treatment. The finished products have been well-accepted on the commercial market.

Many companies have developed and are experimenting with direct steam injection systems for UHT heating. One of the most widely publicized is a European process known as Uperization (8, 15). However, most manufacturers in the United States have heaters and auxiliary equipment whereby comparable treatments and results can be obtained. A typical process might be one where the milk or product is preheated to 165 to 190° F. in a plate heat exchanger, deaerated, heated by direct steam injection to the desired temperature, held, cooled by flash evaporation to approximately the preheating temperature, homogenized, and then cooled by regeneration and ice water to the packaging temperature.

Some of the earlier work on rapid heat processing of fluid foods by steam injection was done by Brown and associates (1) at Albany, California. Their primary purpose was to improve methods of processing fruit and vegetable products. However, the system was also tested on milk.

Burton and associates (3, 4, 5, 6) in England have published several papers on the performance of a UHT plate pasteurizer at sterilizing temperatures. The experiments were concerned

primarily with the efficiency of destruction of bacterial spores of *Bacillus subtilis* and *Bacillus stearothermophilus*. No data were presented on the quality of milk heated to 275° F. In fact, the bacteriological data were obtained mostly on water suspensions of spores. Research is under way in the United States on plate-type exchangers for UHT pasteurization, but no data have been published to my knowledge.

BACTERIOLOGICAL EFFECTS

UHT pasteurization prolongs the shelf-life of dairy products primarily by reducing the vegetative bacterial cells to such a low number that a longer time is required for them to increase in sufficient numbers to alter the milk. Also, the cells may be affected to such a degree that they do not reproduce as rapidly. Kaufmann (14) reported that exposure of MS102 organisms to temperatures ranging from 190 to 205° F. altered the growth and reproductive processes so as to require a 96-hr. incubation period as compared with the standard 48-hr. period.

Hedrick (10) pasteurized raw milk ranging in count from 12,400 to 434,000 per milliliter in a steam infusion heater. Temperatures began at 165° F. and increased at 20-degree intervals from 180 to 300° F. The total holding time was calculated to be 43.8 sec. The approximate time at the highest temperature was 11.2 sec. Plate counts on the milk pasteurized at 200° F. and above ranged from 136 per milliliter down to 2 per milliliter. Generally, the counts were lower after seven days of storage at 40-42° F. Those samples that were pasteurized at 260° F. and above showed no measurable bacterial increase in 14 days.

Milk processed at North Carolina State at 20-degree intervals from 200 to 300° F. for approximately 2 sec. kept for 30 days in glass bottles in a 40-degree refrigerator without detectable flavor deterioration. Other samples heated at 280 and 300° F. for 2 sec. and stored in metal cans at 35 to 38° F. were of commercial quality after 2½ to 3 mo.

Burton and associates (5, 6) studied the effects of UHT pasteurization on spores of *B. subtilis* and *B. stearothermophilus*. Operating temperatures ranged from about 266 to 280° F. They reported that there is a marked inhibition of the germination and/or growth of *B. stearothermophilus* TH24 spores in whole milk treated by the UHT process. This inhibition was common to spores that survived the process and to those that had been heat-activated. Litmus milk autoclaved at 248° F. did not have this inhibitory effect. They also reported that out of each million surviving spores, only about one germinates and grows in UHT sterilized milk.

Brown and associates (1) reported that no surviving organisms were found in samples of milk processed by steam injection at 185° F. for 0.4 sec. and plated at 1:100 dilution. The original count was 25,000 organisms per milliliter, some of which were viable coliform bacteria.

Some experiences of dairy plants have come to my attention whereby heat treatments to 195 to 205° F. resulted in milk of excellent shelf-life but when temperatures of 210 to 230° F. were used, there was a rapid deterioration in keeping quality. In other instances, temperatures of 220° F. and above have been satisfactory. There is not sufficient information to explain the reasons for these differences. Some possible factors that may be involved are (1) the microflora of the milk in the dairy plants from different areas may be vastly different, (2) the total heat treatment as affected by the equipment and particular system may not be comparable, and (3) the procedures and heat treatments may be sufficient to activate the bacterial spores that are present. Normally, bacterial spores are present but we are not aware of them because they go through normal pasteurization without being activated. These spores do not grow when the milk is plated for bacterial counts and do not stain by the techniques employed in making direct counts.

UHT pasteurization is new, and many of the bacterial problems to be overcome have not been experienced. However, it is my opinion that no one should install a UHT pasteurizer expecting to use raw milk that may be of inferior quality or to solve an unsatisfactory cleaning and sanitizing problem. Experience has shown that as demands are made for improved flavor and shelf-life in fluid milk, the improvement must begin at the farm and continue throughout the entire operation.

CHEMICAL EFFECTS

It was indicated previously that there are reasons for UHT pasteurization of dairy products other than bacteriological destruction and extended shelf-life. Some of these are (1) improving the flavor and body of fluid milk, (2) getting maximum stabilization of milk proteins in ice cream manufacture, and (3) possibly converting the heat processing of milk for buttermilk to a continuous process.

Much research has been published on the changes in the electrophoretic patterns of milk proteins as the result of various heat treatments. However, it has not been possible in many instances to relate the results to the characteristics of the finished dairy products. Recently, there seems to be more interest in relating the effects of various systems and heat treatments on dairy products to the percentage of denatured serum proteins.

Jenness (13) summarized the research done at Minnesota relating to the effect of heat treat-

ment on serum proteins. They used a tubular heat exchanger for much of their work, that required 2 to 5 sec. of heating-up time and 2 sec. or longer for cooling. Our studies at North Carolina have been concerned with UHT pasteurization for fluid milk and ice cream mixes. The steam injection heater is a venturi-type.[2] Milk was preheated to 130° F. in a vat (essentially no denaturing of serum protein took place within 1 hr.) and then heated instantaneously (less than 1 sec.) to the desired temperature, held, and cooled quickly by evaporation to approximately 130° F., with further cooling to 40° F. or below. A comparison of the data on the percentage of serum protein denatured by the various systems is presented in Table 1.

TABLE 1

A comparison of heating systems on the denaturation of serum protein

	Temperature	Time	Serum protein denatured
	(°F.)		(%)
Batch	143	30 min.	10
UHT	180	4–5 sec.	10
Minnesota	180	30 sec.	10
Batch	175	30 min.	75
UHT	300	40 sec.	75
Minnesota	300	12 sec.	75

It is interesting that a much longer holding time was required to denature 10% of serum protein by the tubular heat exchanger at 180° F. than by the direct steam injector. However, the reverse is true at 300° F. While it was not possible to compute data for comparable heat treatments from the paper by Hedrick (10), he was able to obtain approximately 86% serum protein denaturation by his system at a temperature of 300° F.

It is recognized that these results are not strictly comparable, because of differences in milk supplies, methods of heating, total heat treatments, and possible differences in methods of analyses. However, these data indicate the approximate temperature and time required to bring about a certain percentage of denaturation of serum protein. More detailed data on the venturi jet heater are presented in another paper at this meeting.

Of perhaps more importance to the fluid milk industry, would be data on the sulphur compounds that are liberated, because these are related to the flavor of the products. Some data are available on vat and tubular heat exchange methods, but very little has been published on steam injection systems. We have initiated some research on these heat-produced changes in the proteins and hope to relate the results to the flavor of the finished products.

[2] Manufactured by Cherry-Burrell Corporation.

OTHER CONSIDERATIONS

Flavor. It is very difficult to evaluate flavor judgments between research studies because conditions of treatment and methods of flavor analysis are so different. Most workers report that they have heated milk in the range of 250 to 300° F. with only slight modifications in the flavor of the finished product. The cooked flavor that was produced disappeared in a short time and the product had extended shelf-life.

We have heated milk at temperatures ranging from 200 to 300° F. at 20° intervals for a calculated hold of 2 sec. The flavor of milk treated at 200 and 220° F. was best, immediately, with the other samples having a reasonably strong cooked flavor. After three days, all of the samples scored approximately the same and were considered very good milk. Shelf-life was excellent for 30 days.

It was not possible to detect body differences as a result of heat treatment.

Many mixes have been heated at temperatures up to 300° F. for a calculated hold of 30 sec. with no flavor problems in the finished ice cream.

Stabilizer replacement. Several UHT systems have been sold to ice cream manufacturers on the basis that there could be a considerable savings in the amount of stabilizer required. Mitten (17) summarized some of this work in a paper presented in 1958. He reported that UHT pasteurization of ice cream mix assures greater bacterial kill, greater water binding characteristics requiring less stabilizer, slightly more dryness when frozen, greater stability against oxidation, and excellent flavor. It is not my intent to get into an extended discussion on this point but rather to present some of the problems that prevent the giving of simple answers.

It is generally recognized that high viscosity in a mix tends to mask flavors. Therefore, for best flavor, one would tend toward heat treatments and stabilization that would produce low viscosity. However, it is desirable that ice cream have a high resistance to heat shock or iciness. Usually, high levels of stabilization retard heat shock. High heat treatments alone do not give the protection to heat shock that is needed in most markets. It may be possible, however, to reduce the stabilizer needed between 25 and 50% in certain flavor-of-the-month specials and other rapid turnover flavors.

There is insufficient published information on specific stabilizers and their behaviour in UHT processes for one to make definite recommendations. Obviously, more research needs to be done on these problems.

As most ice cream manufacturers know,

there are many factors such as the composition and type of ice cream desired, source and type of ingredients used, flexibility and economy of the process and storage qualities needed, that influence the selection of the heating and processing system. Thus, it becomes a matter of selection and adaptation to an individual plant operation rather than to say that any particular process is best.

Other products. Reportedly, other fluid milk products such as skimmilk, chocolate milk or drink, and half and half are being processed satisfactorily in UHT systems. Theoretically, there is no reason why these products should not process as readily as homogenized milk. Sterile egg nog mix is available commercially. It has a lower viscosity than most fresh products but the flavor is acceptable.

Whipping cream is the product with which the most difficulty can be expected. Steam injection and high pressures, velocities and temperatures cause plugging, reduced viscosity, and some reduction in whipping ability.

UHT processing is still very new and it is reasonable to expect that as more knowledge and experience are obtained, the process will have a wider use and adaptability.

SUMMARY

UHT pasteurization will result in longer shelf-life to fluid milk without serious damage to the flavor. In some instances, the flavor may be improved.

The quality of the raw milk must be excellent, in order to obtain the greatest benefits from the UHT process.

Post-pasteurization contamination is probably more important because of the increased time that the milk is held prior to consumption.

UHT pasteurization will give added stabilization to milk proteins in ice cream mix but this alone will not give the amount of protection to heat shock that most plants need. Some of the systems that encompass vacuum treatment will enhance the flavor of the mix.

The flexibility, adaptability, and economy of UHT pasteurization is still primarily an individual plant problem.

Further research is needed to answer many of the problems confronting the widespread use of UHT methods in the dairy industry.

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IMPRESSIONS OF RUSSIAN DAIRY FARMING [1]

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A party of seven livestock specialists from the United States had the unusual opportunity of spending 5 wk. in the Soviet Union, in the summer of 1959, to study the livestock industry. This was one of the agricultural teams of the Cultural Exchange Program in effect between the United States and the Union of Soviet Socialist Republics. The members of this team were E. E. Heizer, Head, Department of Dairy Husbandry, University of Wisconsin; L. E. Hanson, Head, Animal Husbandry Department, University of Minnesota; A. D. Tillman, Professor, Animal Husbandry Department, Oklahoma State University; A. V. Nalbandov, Professor, Animal Husbandry Department, University of Illinois; Wesley Keller, In Charge, Range Investigations, Forage and Range Research Branch, Farm Crops Research Division, ARS, USDA; C. E. Terrill, Chief, Sheep, Goat, and Fur Animal Research Branch, Animal Husbandry Research Division, ARS, USDA; and R. E. Hodgson, Director, Animal Husbandry Research Division, ARS, USDA, the team leader.

The purpose of this paper is to give a brief general description of the authors' impressions of dairy farming in the Soviet Union as they observed them. This includes observations relating to cattle and production methods used on collective and state farms and observations on the research and education programs that support the dairy farming industry. It will be said at the outset that although the group did spend 5 wk. observing Soviet dairying, they do not pose as experts on the subject. Among other things, the language barrier made it difficult to obtain the fullest possible information. They do have some impressions, however, that are recorded here for the use of Journal readers.

STUDY TOUR IN THE U.S.S.R.

The study tour began in Moscow, where nearly a week was spent in meetings with specialists in the Ministry of Agriculture and visiting the All-Union Academy of Agricultural Science (Timiryazev), the Animal Husbandry Research Institute, and the Academy of Genetics. These are all national institutions concerned with livestock production problems in all the 15 Republics. A collective farm at Gorky, near Moscow, was also visited. The next stop was in the Leningrad area, where the group visited the All-Union Academy of Sciences Institute of Zoology; the Pavlov Institute of Physiology; the Pushkin Academy of Agricultural Science and Laboratory for Animal Multiplication; a large dairy processing plant, and a collective farm. The next area for study was the Latvian Republic, where near Rega the group visited the Latvian Institute of Animal Husbandry and its experimental farm; the Latvian Ministry of Agriculture and two collective farms.

From Rega the group traveled to Kiev, the capital of the Ukraine Republic. Visits were made to the Ukrainian Ministry of Agriculture, the Animal Husbandry Research Institute and its state farm at Teresino. The next stop was Kharkov, where a visit was made to another Ukrainian Animal Husbandry Research Institute and its State Farm for Animal Multiplication; the Ukrainian Research Institute for Poultry and a collective farm. From Kharkov the group traveled to the arid southern Ukraine to visit the Askaniya Nova Agricultural Research Institute and its State Farm for Pedigree Breeding. In this region visits were made to one state and two collective farms.

The next region of study was the Stavropol Department of the Russian Republic north of the Caucasus Mountains, another arid region. Visits were made to the local Ministry of Agriculture, the All-Union Research Institute for Sheep Breeding, the Stavropol Agricultural Academy, and a collective farm.

The next stop was in the southeastern part of the Kazakh Republic at Alma Ata along the foothills of the Ala Tau Mountain range. Visits were made to the Kazakh Ministry of Agriculture, the Kazakh Academy of Agricultural Science, Institute of Animal Husbandry, and two collective farms. From Alma Ata the group traveled to Rubtsovsk in the Altai Department of the Russian Republic. This is in the so-called new lands area of Siberia.

The study tour took the group into most of the important livestock producing regions. This included the humid nonblack soil area along the Baltic, the black soil and semiarid and arid Ukrainian Steppe regions, the arid regions north of the Caucasus Mountains, and the arid and semiarid regions east of the Urals. Cattle raising and milk production figure prominently in the farming scheme in all of these areas.

[1] This paper is taken in part from Livestock in the Soviet Union, published by the U. S. Department of Agriculture, Washington, D. C., 1961. The reader is referred to this report for a fuller discussion of the livestock industry of the U.S.S.R. in 1959. For the convenience of the reader, all figures have been converted to U. S. usage by appropriate conversion factors.

ORGANIZATION OF FARMING—COLLECTIVE AND STATE FARMS

Russian agriculture is organized in state and collective farms. There are no family farms as we know them. There are about 60,000 collective and 6,000 state farms. They were first organized in the 1930's by obligatory consolidation of the holdings of more than 20 million former independent peasant farmers. There were many more collective farms in the 1940's and 1950's, but the numbers have been reduced by consolidation into larger farms and the formation and increase in the number of state farms. When collectivization of the farms took place, the peasant owners were allowed to keep small plots (about one to two acres in size) where they could have their homes and grow garden crops and maintain a few privately owned animals.

The former members of the collective have a residual claim to the income of the collective, based on the kind and amount of work they perform. However, the Soviet State, which controls the collectives, has a prior claim to its share, which may reach half or more of the produce of the collective. In contrast, the state farms are owned and managed by the state and the workers are paid wages. The emphasis is not only on larger collective and state farms but also in the direction of fewer of the former and more of the latter. All but about 3 to 5% of the 480 million acres of land in crops are under control of the state and collective farms, the remainder being taken up by private garden-plot-homesite holdings. Even so, the peasants living on these small privately owned holdings account for a significant part of the livestock population. It was stated that over half of the milk production and about 40% of the meat presently come from privately owned livestock. The private sector owns about 40% of the cattle, one-third of the hogs, and one-fourth of the sheep and goats.

The animal husbandry study group visited 15 state and collective farms while in the Soviet Union. All of the farms visited carried on major livestock enterprises, but the farms with few exceptions were in no way confined to livestock production. Usually cattle, pigs, and/or sheep were major livestock enterprises. Chickens often were an important project. Coupled with this, and the production of feed crops such as forage, corn, and other feed grains, the growing of wheat, sunflowers and sugar beets, where they could be grown, also were major enterprises.

Each farm is under the supervision of a chairman, on state farms appointed by the state, and on collective farms elected by the farm membership. The chairman must meet the approval of the state and he can be removed by the state. On the collective farms it was found, in some cases, that a committee of several individuals chosen by the members served with the chairman in planning the farm program and activities. In this planning, full consideration must be given to the quotas for the farm assigned by the Ministry of Agriculture. This applies both to the kind and amount of various crops and products assigned to the farm to produce.

Each collective or state farm employs a staff of technicians, usually college graduates, to advise, direct, and supervise to a degree the production methods and practices. Depending on the size and variety of enterprises, these may include one or several agronomists, zootechnicians, veterinarians, soils specialists, horticulturists, etc. These people are depended on for professional advice and with the chairman are the key liaison between the farm and the Ministry officials and the institutes. In this respect they may be considered as similar to our county agent service, except that they are more specialized and confine their efforts to a particular farm.

The farm work is organized according to enterprises, such as caring for cattle, growing field crops, gardening, etc. The workers are organized into brigades, with a leader at the head of each. Workers, both men and women, have some choice in the kind of work they would like to do. Children also work in various capacities on the farms. The pay received depends on the job classification and work output in comparison to the work-norms established by the management, provision being made for additional pay for work performance above the work-norm. This appears to be a great incentive for extra effort and apparently is important to the farm organization in meeting the production goals set for it.

Payment for work on collective farms is either in produce or in wages, and on state farms in wages. Wages are low according to American standards. While pay varies according to kind and amount of work on a particular farm, it also varies from farm to farm and from region to region. Average workers on farms visited received about $40 to $110 per month. In many families the man and his wife and the older children all work.

The facilities on most farms visited left much to be desired. Varying degrees of modernization were evident. Building materials were apparently quite limited. Much of the construction was of the mud block adobe type. Housing for cattle probably was better than for other classes of livestock. Most of the barns had good concrete floors, gutters, and mangers, wood or metal stanchions and, in many, water cups were found. Some had wooden paddle barn cleaners; others had litter carriers on tracks. Milking by machine was observed more often than was hand milking.

CATTLE BREEDS

The cattle of the Soviet Union are dual-purpose, but the major emphasis up to this time has been given to milk production. With very few exceptions there are no separate and distinct breeds of beef cattle at the present time. There was evidence, however, that efforts are currently being given to developing specialized breeds for meat production. The improvement in feed supplies may account in part for the feeling that specialization can not take place. In the spring of this year several hundred head of beef breeding stock were imported from the U.S. to the U.S.S.R.

There are 28 described breeds of cattle in the Soviet Union. A brief description of each of these breeds, including the principal color markings and the minimum requirements for size and milk production at specific stages of life for admittance to the First Class Registry, is shown in Tables 1 and 2. Only one of these,

TABLE 1

Description of Russian cattle breeds

Breed	Foundation stock used in origin	Approx. date of origin	Origin and areas of popularity in U.S.S.R.	Type	Principal color markings
1. Kolmogora	Native, Friesian	1750	Central, North, West Central	Milk-meat	Black, white; red, white, spotted
2. Tagil	Native, Kolmogora, Dutch, Yaroslav	1760–1800	Middle Urals	Milk-meat	Black, black spotted, red; red spotted
3. Yaroslav	Native	1850–60	Central, North	Milk-meat	Black or red with white, white around face, eyes and underline
4. Istobensk	Native, Kolmogora, Swiss, E. Friesian, Yaroslav	1924–25	North Central	Milk-meat	Black or red and white spotted
5. East Friesian	Imported from Germany	1700	West Central	Milk-meat	Black and white
6. Aulieatinsk	Native, Dutch imported	1882–1912	Khirgiz, Kazakh Repub.	Milk-meat	Black or red spotted
7. White Faced Ukraine	Native, Dutch imported	1780–1800	Central Ukraine	Milk-meat	Black, red or chestnut with white face
8. Red Steppe	Native, East Friesian	1910–11	Southern Ukraine, Central Asia, South Central	Milk-meat	Red—light to dark
9. Red Estonian	Native, Red Dane, Swedish Red	1900	Estonia	Milk-meat	Red
10. Latvian Brown	Native, Red Dane, Swedish Red	1860	Latvia, Central USSR	Milk-meat	Red, chestnut
11. Red Lithuanian	Native, Ayrshire, Swiss, Friesian, Red Dane		Lithuania	Milk-meat	Red of varying shades; red spotted
12. Black Spotted Lithuanian	Native, Dutch imported, Swiss	1930–35	Lithuania	Milk-meat	Black, white spotted
13. Black Spotted Estonian	Native, Dutch imported	1900–20	Estonia, N. Central USSR, Northwest USSR	Milk-meat	Black spotted
14. Kostroma	Native, Swiss imported	1917	Central	Milk-meat	Light or dark grey, brown
15. Simmental	Imported from Switzerland	1800	Ukraine, W. Central, USSR	Milk-meat	Yellow or red spotted
16. Sichevski	Native, Simmental	1930	W. Central, Central, Siberia, White Russia	Milk-meat	Pale yellow or red spotted
17. Swiss	Imported from Switzerland	1862	Central	Milk-meat	Brown
18. Red Humpback	Native, Tyrolean	1860	Central USSR	Milk-meat	Red

TABLE 1 (Concluded)

Breed	Foundation stock used in origin	Approx. date of origin	Origin and areas of popularity in U.S.S.R.	Type	Principal color markings
19. Kurgan	Native, Shorthorn imported	1900	Central Urals	Milk-meat	Red or red spotted
20. Lebedin	Native, Brown Swiss	1934	Central Ukraine, W. Central	Milk-meat	Grey, brown
21. Ala Tau	Native, Brown Swiss	1900	South Asia Republics	Milk-meat	Brown
22. Bestuzhev	Native, Shorthorn	1850–1900	East Central, Urals	Milk-meat	Red, red spotted
23. Yurin	Native, Tyrolean, Swiss	1900	Central USSR	Milk-meat	Red, brown
24. Red Tambov	Native, Tyrolean, Simmental	1900	Central USSR	Milk-meat	Chestnut, red, red spotted
25. Shorthorn	Imported	1900	Central USSR, Central Urals and Northern Caucasus	Meat-milk	Red, white, roan
26. Kalmitz	Native	1917	Northern Caucasus	Meat	Red, red spotted
27. White-faced Kazakh	Native, Hereford	1930	Caspian, South Asia	Meat-milk	Red with white face
28. Grey Ukraine		1910	Ukraine, Kuban	Meat-milk	Grey

TABLE 2

Minimum requirements for the various breeds for admittance to first class registry

Breed	Live weight		Milk production—300 days			
	Bulls, 5 yr. old and over	Cows at 3rd calving	First lactation	Second lactation	Third lactation	Butterfat average
	(lb.)					(%)
1. Kolomogora	1,760	1,056	5,060	5,940	6,600	3.7
2. Tagil	1,650	1,012	4,840	5,720	6,380	4.0
3. Yaroslav	1,584	968	4,620	5,500	6,160	4.0
4. Istobensk	1,650	1,012	4,620	5,500	6,160	4.0
5. East Friesian	1,804	1,100	5,720	6,600	7,480	3.6
6. Aulieatinsk	1,584	946	4,620	5,500	6,160	3.8
7. White-faced Ukraine	1,650	968	4,884	5,720	6,380	3.7
8. Red Steppe	1,650	1,034	5,060	5,940	6,600	3.7
9. Red Estonian	1,650	1,034	5,060	5,940	6,600	3.8
10. Latvian Brown	1,650	1,034	5,060	5,940	6,600	3.7
11. Red Lithuanian	1,650	1,034	5,060	5,940	6,600	3.7
12. Black Spotted Lithuanian	1,804	1,100	5,500	6,380	7,260	3.7
13. Black Spotted Estonian	1,804	1,100	5,500	6,380	7,260	3.7
14. Kostroma	1,826	1,122	5,720	6,600	7,480	3.8
15. Simmental	1,804	1,122	5,060	5,940	6,600	3.7
16. Sichevski	1,848	1,144	5,280	6,160	6,820	3.8
17. Swiss	1,760	1,078	5,280	6,160	6,820	3.7
18. Red Humpback	1,540	946	4,400	5,280	5,940	4.0
19. Kurgan	1,694	1,078	4,840	5,720	6,380	3.9
20. Lebedin	1,760	1,100	5,280	6,160	7,040	3.8
21. Ala Tau	1,760	1,078	5,060	5,940	6,820	3.8
22. Bestuzkev	1,716	1,056	4,400	5,280	5,940	3.8
23. Yurin	1,540	946	4,400	5,280	5,940	4.0
24. Red Tambov	1,760	1,100	5,060	5,940	6,600	3.7
25. Shorthorn	1,760	1,100	4,400	5,280	5,940	3.8
26. Kalmitz	1,650	1,056	.....	.....	.....	....
27. White-faced Kazakh	1,640	1,060	3,080	3,740	3,960	3.8
28. Grey Ukraine	1,694	1,100	3,300	4,180	4,840	4.1

the Kalmitz, is classed as a meat breed. In addition, three breeds of Zebu are recognized. One breed of black-colored Yak and one breed of black or dark grey or brown-colored buffalo is also recognized.

CATTLE AND MILK PRODUCTION STATISTICS

The cattle population in 1958 was reported to be 66.8 million head, of which 31.4 million were cows.[2]

A recent report from the USDA [3] describes developments in milk production. The essential data in this report are summarized in Table 2. These data show a very large increase in total production of cow's milk, 62.5% from 1940 to 1957. This compares with an increase of 15.1% in the U. S. in the same period. The report suggests serious doubt concerning the validity of the official milk production figures. It suggests that a more realistic figure for the 1957 production would be about 100 billion pounds rather than the 117.5 billion pounds reported. The report also shows that the numbers of cows had increased by 42.7% from 1940 to 1957, while the number of cows and heifers 2 yr. old and over kept for milk in the U.S. had decreased by 8%.

The average production of milk per cow has shown a significant increase. A comparison of the per-cow production, as calculated from the data in Table 3, by years, is shown in comparison with that of U.S. cows in Figure 1. According to these data, production per cow in the U.S. from 1940 to 1957 had increased by 36.9%, and the production per cow in the U.S.S.R. increased 24.8%. In the latter case,

[2] U.S.S.R. in Figures—A Statistical Summary—Central Statistical Department, Ministry of Agriculture, Moscow. 1958.

[3] Milk Production in the Soviet Union—Recent Developments—Volin, Lazar, Foreign Agricultural Service, FAS-M-58, May, 1959. U. S. Department of Agriculture, Washington, D. C.

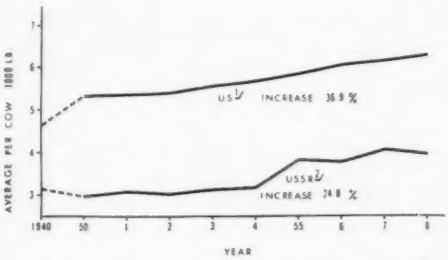

FIG. 1. Trends in milk yields per cow in the United States and in the Union of Soviet Socialist Republics.

[1] Agricultural Statistics Series, U. S. Department of Agriculture, Washington, D. C.

[2] Milk Production in the Soviet Union, FAS-M-58, U. S. Department of Agriculture, Washington, D. C., 1959. Average yield per cow calculated by dividing figures for all cows, Column 1, Table 3, into figures for total yield for cow's milk, Column 1, Table 1.

a very significant increase took place in the year 1955. Previous to that year, and following that year, the year-to-year increase was very small. Perhaps this large increase could be the result of a change in statistical reporting rather than an actual increase in per-cow milk production.

The best estimate that could be arrived at is that the average production of milk per cow is about 4,000 lb. There apparently is a great range in production per cow in different areas and on different farms in the U.S.S.R., just as there is in the U.S. As an example of this, the average milk yield per cow reported for each of the state and collective farms that the study group visited was 8,147 lb., with a range from 15,180 to 2,236 lb. Average for all cows in the Latvian Republic was 5,500 lb.; for the Stavropol Department 5,940 lb.; and for the

TABLE 3

Total cow numbers and milk production by farming sources in the U.S.S.R. for selected years [a]

Year	Cow's milk production: Total [b]	Collective farms	State farms	Private sectors	Per cent of production coming from: Collective	State	Private	All cows [c]	Av. yield per cow [d]
	(billion lb.)				(%)			(number, million)	(lb.)
1940	72.3	12.0	4.3	56.0	16.6	6.0	77.4	22.8	3,173
1950	73.6	14.3	4.5	54.8	19.4	6.2	74.4	24.6	2,992
1953	75.5	19.6	6.5	49.5	25.9	8.6	65.5	24.3	3,109
1955	90.7	29.6	7.6	53.5	32.7	8.3	59.0	26.4	3,810
1957	117.6	43.5	12.1	62.0	37.0	10.2	52.8	29.0	4,054

[a] Adopted from Tables 1, 2, and 3, FAS-M-58-USDA.

[b] An additional three to four million pounds of milk are reported from sheep and goats; 1958 production given as 127.4 million pounds, of which probably three million are from sheep and goats.

[c] Reported as all cows.

[d] Derived by dividing total cow milk production by total of all cows.

collective and state farms in the Ukrainian Republic 4,950 lb.[4] It is apparent that the study group was given the opportunity to see, by and large, the better-producing herds on their trip. At the same time, it is not difficult for a visitor to have the same experience on a tour to study dairy farming in the U.S.

The study group had little opportunity to observe livestock production practices carried on by the private land-holders on their one-acre plots. This is unfortunate when it is realized that more than half the milk and 40% of the beef are reported to come from these private holdings. What the group was able to see in this respect indicated that conditions were not particularly conducive to high production per animal unit.

Evidently not much attention is given to type, at least nowhere near the attention that is given to it in this country. If there is, the ideas of type are quite different than ours. The cows are coarse, rough, often low in the back and rump, and the udders are not well balanced. Individuals of the various breeds are lacking in uniformity, both in color and appearance. The main emphasis is on the utility aspects, such as size and weight for age and on milk yield and the butterfat content of the milk.

CARE AND MANAGEMENT

Herds on collective and state farms are large. Even so, the general practice appears to be to divide them into smaller units and to keep milking cows together and away from dry cows and young stock. Barns usually hold about 100 milking cows and there may be several such barns on a farm, depending on the size and importance of the cattle enterprise. In the northern areas, enclosed stanchion barns are in common use the year around, whereas in the southern areas enclosed barns are used in the winter but in summer the cows are often housed in open-sided stanchion barns. These methods appear to be an effort to house producing animals so that adverse climatic factors are minimized. There was no evidence of the use of loose housing and milking parlors. Frequently, milking was done in open yards.

Pasturing is not practiced extensively. Only in Latvia did the study group observe cattle grazing on pasture to any great extent. Here intensive rotational grazing on productive permanent pasture, like one might see in the humid areas of the U.S., was common. This is about the only place where fences in any number were observed. Most generally, the practice throughout other parts of the U.S.S.R. is to keep the producing cows in dry lot the year around and bring the feed to them. Dry stock and heifers seemed less well cared for, are pastured, and are given supplemental feed as needed to keep them in fair condition. With the absence of fences, these animals are tended by herders, generally women.

The Russians believe in exercising their animals. Those not pastured are given exercise by being driven several miles each day. The group was told by the Head of the Cattle Department of the Timiryazev Academy that exercise improves the ability to digest food, does not increase the nutritive requirement, and enables cows to produce more milk per unit of food. Exercising animals, therefore, appears to be a regular part of the routine in caring for cattle on collective and state farms.

The animals observed appeared to be well cared for. Most often women care for the animals and they apparently have a love for cattle and give them much close attention. Animals, even herd bulls, seemed to be very docile in response to this kind of treatment.

MILKING PRACTICES

It is the usual practice to milk cows three times daily. Frequently, with the higher producing cows, milking is done four and even five times daily, particularly in the early part of the lactation period. On the farms visited we observed good milking practices. This included cleaning the udder and teats before milking, using a strip cup, conditioning the animal, and then milking by machine or by hand. The milk from each milking is measured in a specially designed measuring bucket and the volume recorded. Samples are taken for butterfat determinations on one or two days each month. Butterfat determinations are made by the Gerber method. The study group was told on a number of occasions that mastitis was not a serious problem, since it is largely avoided by giving close attention to good milking practices.

The group could not determine how extensively machine milking was used, but our impression is that considerable effort is made on collective and state farms to adopt this method of milking. Most of the farms visited were using milking machines. It is very doubtful that any of the large numbers of cows on private holdings are milked by machine. Considerable attention apparently is given to improving milking machines. The Animal Husbandry Research Institute at Kharkov demonstrated a portable four-unit milking machine they had recently developed. It was suspended on a carrier track back of the cows and could be moved along the string from one end of the barn to the other. It had individual compartments to receive the milk from four cows, each

[4] The study group made a number of inquiries concerning the method by which the average production of all cows is computed by various Ministries of Agriculture. They were informed on one occasion that the average production per cow, as measured on collective and state farms, was projected for that part of the cow population privately owned (approximately 53%).

with a window and a measuring device to measure the amount of milk. The milk could be drained from this to a receiving can. The machine claws, cups, etc., looked much like the ones used on our machines. This milker was apparently working well but it looked crude and as if it would be difficult to clean.

This machine was observed in the Animal Husbandry Department at the Moscow Permanent Exhibition where it was being demonstrated. The machine utilizes what was called a special three-phase action which was claimed to do a faster and more complete job of milking than the conventional two-phase units. The principle utilized 45 to 50 pulsations, involving 60% of the time in suction, 20% in pressure, and 20% in release. The vacuum was 400 mm. of mercury. Each unit will milk 40 cows per hour.

Milk was strained through cloth from the bucket into receiving cans in the barn and the cans were stored in water tanks or, more often, transported soon after milking to the receiving station or factory. There was little evidence of refrigeration storage and no evidence of refrigerated bulk storage tanks on farms. Likewise, there was no evidence of steam sterilization facilities for sanitizing milk utensils on the farm, and frequently the water supply appeared to be limited. The availability of veterinary service on most farms no doubt aids in the sanitary aspects of milk production.

CALF RAISING

Observations made indicated that methods of raising young calves were good. Again, the availability of lots of labor and love of animals affords individual attention so necessary for successful calf raising. The methods used are not unlike ours. The calves are taken from their mothers two or three days after birth and after receiving the benefit of the colostrum, are placed in individual pens or in small groups. Some of the individual pens were of the raised-floor type. Calves receive whole milk for 2 or 3 wk. and skimmilk for 4 to 6 mo. Green feed or hay is offered as soon as calves will take it. A grain mixture also is offered and fed in moderate amounts until the calves are old enough to sustain themselves in reasonably good condition on forage. Antibiotics were used on some farms. Generally, simple grain mixtures or single grains such as oats or barley were used. Not much use of milk replacers or starters was observed, probably because most farms have plenty of liquid skimmilk available.

FEEDS AND FEEDING PRACTICES

Much emphasis is placed on roughage in feeding cows. In the northern humid regions cows are pastured on good permanent pasture under intensive rotational grazing. In other areas, cows are kept in and green feed is fed in barns or in feeding yards. Several different forage crops, such as grain crops, Sudan millet, sweet clover, alfalfa, corn, etc., are fed in season throughout the summer. Hay in limited amounts, but lots of corn silage and straw, are fed in winter. A grain mixture containing about 15% protein is fed to producing animals at the almost universal rate of 1 lb. daily to each 3.5 to 4 lb. of milk produced. Feedstuffs making up the grain mixture are various combinations of several of the following, depending on availability: corn, barley, oats, mill feed, beet pulp, beet molasses, sorghum, and oil meals from flaxseed, cottonseed, and sunflowers. The corn silage is made from corn with few if any ears (in northern latitudes), or corn with the grain just forming on the cob. The practice is to harvest the corn early to obtain more protein in the leaves. The corn is grown thick, and the growing season is short, which may be another reason for early harvesting. Much of the corn silage is made in trenches, bunkers, or stacks. Much of it observed was rather sour and only of average quality.

BREED IMPROVEMENT PROGRAMS

The breed development and improvement programs are handled by the Ministry of Agriculture through the various institutes and academies. A number of different institutes visited

FIG. 2. Dairy barn—Red Partisan Collective Farm—near Leningrad.

FIG. 3. Street milk dispenser—Stavropol.

had organized improvement programs in progress with collective and state farms for the breeds that they were interested in. These programs may be different in the different republics. Since all of the collective and state farms regularly record the volume of milk produced, and presumably most of them make butterfat determinations for individual cows in their herds, data on a large percentage of the cows are available for evaluating breeding material. It was not made clear how effectively these data are used for this purpose. It was not evident that a wide-scale sire evaluation and proving program was in progress. However, in Latvia it was stated that 80% of the cows were on organized record of performance programs. The group was shown an elaborate system of forms that was just being adopted for data recording, analysis, and sire evaluation in the Latvian Republic. On close inspection, it was apparent that these forms were almost an exact copy of the new forms recently developed for similar work by the dairy extension workers at Cornell University in cooperation with the USDA.

At the Animal Husbandry Research Institute at Kharkov the group was told that this Institute has the responsibility for sire evaluation work for all breeds for the Ukraine Republic. The breed improvement work is based largely on sire selection. Some work had been done before the war and they are just now getting back into this work in an organized way. In relation to sire proving using daughter-dam comparisons, the man in charge indicated that the results are confounded by the steadily increasing level of feeding that has been taking place in recent years. For this reason, he stated, the sire evaluation work is based on both the daughter-dam comparisons and on the daughter averages, with contemparary comparisons of nonrelated animals in the same herd producing at the same time. With contemporary comparisons, milk yield, butter test, and yield and weight of animal are considered. The productivity of a bull's daughters is expressed as a percentage of the productivity of the mates and of the mothers and the mothers' mates.

Pedigree evaluation is also used to judge the merits of sires. This is used when insufficient information is available on production of daughters of bulls.

It was stated that presently only about 25% of the bulls in artificial breeding studs had been selected on one or the other of these methods, mainly the pedigree evaluation method. It is planned to expand these methods of sire evaluation as much as possible, especially for bulls in artificial breeding.

In addition to the sire evaluation work, research institutes operate pedigree increase farms where breeding animals of one or another breed are raised and distributed to other farms or placed in artificial insemination studs operated by the institutes, or in other studs.

FIG. 4. Dairymaid milking with machine—Genetics Experimental Base State Farm—Gorky.

The herd books for the various breeds are operated by the institutes. There apparently has been considerable emphasis given to the pedigree program on both state and collective farms. In 1932, 10% of the cattle on state and collective farms were pedigreed. In 1939, the percentage was 29, and in 1955 it was 74, with a total of 19.2 million head.[5] The qualifications for admittance to the pedigree classes are not well defined. Animals are recorded in the herd books under several classes of entry, upon meeting the requirements set and upon passing an inspection conducted by an inspection committee established for this purpose.

An animal apparently is considered a purebred and may become a pedigreed animal if it is the result of three or four crosses to a pedigreed bull of the breed and meets the standards set by the Ministry of Agriculture. These standards apparently are based on live weight at a given age, productivity, and origin (breed characteristics). The herd book pedigree program apparently is progressive, in that animals progress according to percentage of pure breeding into the purebred class. In addition, there are provisions for three classes—elite, first class, and second class animals—that animals may qualify for, depending on how well they meet the standards set by the Ministry. Animals under consideration for qualifying are inspected by an inspection committee of representatives from the Ministry and the Institute.

It is apparent that this must be a very active program, in that in all classes of livestock the number and the percentage of all collective and state farm–owned livestock have increased very rapidly. Pedigreed animals constitute a much larger percentage of the livestock in the U.S.S.R. than in the United States. It is obvious, however, that the herd book programs of the two countries differ greatly.

BREEDING RESEARCH

Extensive breeding research with cattle is in progress at all of the animal husbandry re-

[5] See Footnote 2.

search institutes visited. This work is directed toward developing new breeds especially adapted for particular environments, improving existing breeds in milk yield, butterfat content, and in size. The breeding work perhaps of necessity follows closely the Lysenko theory that all laws of heredity are subordinate to environment. A few examples of the research studies observed will be discussed.

Breeding research at experimental genetics base. The study group had the opportunity to review Dr. T. D. Lysenko's research with cattle at the Experimental Genetics Base at Gorky. Dr. Lysenko subscribes to the Michurian Theory of Biological Inheritance. His research with dairy cattle was outlined to demonstrate this to the group. The primary object of the research is to increase the butterfat content in the milk. The foundation herd is of the large-sized Kostroma breed, giving milk that averages 3.5% butterfat. The plan was to cross these with Jersey bulls imported from Denmark. These bulls apparently did not have tested daughters and he assumed that their transmitting ability for butterfat was 5.5% of the breed average. The offspring resulting from the first cross he reported produced milk with butterfat content of 5.0% or higher (with only one exception). Furthermore, these offspring produced milk in large volume, like the breed of the dams. Dr. Lysenko stated that the 75 bulls resulting from these first crosses, when used on any other cattle, would produce offspring that gave milk testing at least 5.0%. The cows observed by the group, both foundation and crossbreds, were good-looking, productive cows. They lacked nothing in the way of care and feed to provide an excellent environment for them to express their hereditary ability to produce. We observed no control population that he was using and there was lack of evidence of a carefully designed experimental plan. We believe that feeding and management conditions have improved greatly in this herd in the last few years. When data were asked for to substantiate his statements, none was offered.

Dr. Lysenko argued that there was need for milk of a high butterfat content and his experiment proceeded to obtain it. He said that the nutritive requirement per liter of milk is the same regardless of its butterfat content, a statement that the group could not accept. He concluded that he has created in these simple crosses a breed of cattle with characteristics of having small calves that grow into big cows that produce large volumes of milk of high butterfat content and that there will be no segregation in these characteristics. Dr. Lysenko dismissed us by stating that the development of a species is governed by certain laws of biology that need to be studied. This was about the only basis on which the study group and Dr. Lysenko could agree.

Research to develop the Ala Tau breed. One phase of the research of the Animal Husbandry Research Institute at Moscow and the Kazakhstan Academy of Agricultural Science is the development of a breed of cattle that would be particularly adapted to the mountainous and irrigated valley region of southern Kazakhstan. Native cattle were available and the objective was to introduce stock to develop a breed that yielded larger quantities of milk and would have good meat qualities.

Foundation native dams were crossed to Brown Swiss bulls. The female offspring were bred to Brown Swiss bulls producing ¾ Brown Swiss, ¼ Kazakh. The superior animals resulting from the second cross, based on appearance and size (weight for age), were interbred and became known as the Ala Tau. The poorer animals were mated to Brown Swiss bulls to produce ⅞ Brown Swiss, ⅛ Kazakh. Their offspring were bred to the Ala Tau bull and the offspring became the Ala Tau. Intensive selection was practiced, particularly with the Ala Tau bulls used. Selection was based on color, appearance, milk yield, butterfat test, and body size. By using state and collective farm herds, some 30,000 cows were available and intensive selection was practiced. The animals that the study group observed represented the second, third, and fourth generations. They

FIG. 5. Latvian Brown cows, Latvian Animal Husbandry Institute State Farm near Rega.

FIG. 6. Simmental bulls at Artificial Insemination Stud, Animal Husbandry Research Institute, Kharkov.

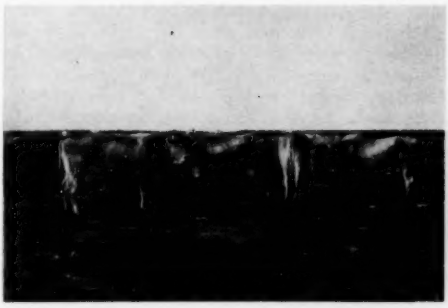

FIG. 7. Lebedin cows, Animal Husbandry Research Institute, Kharkov.

FIG. 8. Heifers of Red Steppe breed—Askaniya Nova Research Institute, Southern Ukraine.

were large, productive animals of good fleshing qualities and they looked very much like our grade Brown Swiss cattle. Again, there was no evidence of the existence of control populations that were used to measure progress being made.

Research with the Red Steppe breed. The Askaniya Nova Research Institute has a major breeding research project with cattle of the Red Steppe breed. Three phases of work are in progress: (1) To improve the Red Steppe breed; (2) to create a new commercial dual-purpose type by crossing the Red Steppe with the Shorthorn; and (3) to improve adaptability and increase the butterfat content of the milk of the Red Steppe by crossing with the Zebu. In addition, this station supervises the breed registry and the breeding policy of the Red Steppe breed. They also operate a state farm for increase in pedigreed Red Steppe breeding animals for distribution to the industry. At this farm three different lines of Red Steppe cattle have been established: (1) High milk yielding line; (2) high butterfat testing line; and (3) high meat line. The establishment of the high butterfat test line, based on the performance of one outstanding cow, was illustrated. The technician in this way demonstrated how the inherited ability for high butterfat percentage in the milk was fixed. It was stated that heritability of a characteristic takes the direction of selection, and in this case the prime consideration in relation to the inheritance of butterfat percentage should be given to the dam.

In the formation of this high test line, a foundation cow, No. 48, is the prime contributor. What she descended from seemed unimportant. Her nine lactations varied from 5,060 to 9,460 lb. of milk and the test varied from 4.5 to 4.8%. She produced two daughters from two different bulls. One of these bulls was from a dam with one lactation, 11,644 lb. of milk testing 3.6%. No information was available on the other dam. The daughter from the latter bull had three lactations that ranged from 5,654 to 9,460 lb. of milk and the test varied from 4.1 to 4.8%. The daughter from the other bull and the foundation cow No. 48 had five lactations that ranged from 7,040 to 8,140 lb. of milk and the test varied from 4.1 to 4.4%. This daughter was bred to a bull from a cow that produced in one lactation 8,000 lb. of milk testing 4.23%. The female offspring from this mating had two lactations, with 6,233 and 6,158 lb. of milk testing 4.28 and 3.93%, respectively. The average test for the herd is about 3.8 to 3.9%.

Five bulls from the foundation cow, her two daughters, and one granddaughter are presently being used in artificial insemination in the state farm herd to establish this high test line. One of these bulls has been proved both on pedigreed cows and on grade cows. The 27 purebred daughters by this bull averaged 3.95% butterfat and their 27 purebred dams averaged 3.68%, an average increase of 0.27. On his proof with grade daughters, their average butterfat test was 3.75% and their dams' average butterfat test was 3.78%, a decrease of 0.03. This was all of the data they could show us to substantiate their claim of the establishment of a high butterfat test line and the mode of inheritance of butterfat.

The cross-breeding work with the Shorthorn is designed to develop a type that includes high milk yield, high butterfat test, and good carcass quality. They are looking for a higher meat-to-bone ratio than is present with the Red Steppe breed, with a lean meat that is more juicy, more tender, and more marbled. They anticipate this will be achieved with a combination of 7/8 Red Steppe and 1/8 Shorthorn.

Breeding research with Simmental cattle at the Animal Husbandry Research Institute, Kharkor. This station is the principal organization doing breeding research with the Simmental breed. They are also working with the Red Steppe and the Lebedin breed which was founded at this station.

The work with Simmental cattle is directed toward increasing the milking ability and increasing the butterfat content of the milk. In the last 12 yr. they have been establishing in

this herd two lines, one a high milk line and one a high fat test line. At present, the high milk line is averaging 18,260 lb. of milk, with an average test of 3.75. The average cow weighs 1,748 lb. (mature) and yields about 1,000 lb. of milk per 100 lb. body weight per lactation.

The high fat line is averaging 12,100 lb. of milk, with an average test of 4.2%. The average cow weighs about 1,430 lb. (mature) and yields about 800 lb. of milk per 100 lb. body weight per lactation.

These lines were established by selection and use of bulls that sired daughters that were either high in milk yield or that had a high butterfat test for use on the respective lines. Considerable line breeding was practiced and, of course, rigid selection was used. The evident purpose is that when these lines are firmly established and fixed, they will be crossed with the anticipation that the crosses will express both the high milk yield and high fat test of the parent lines.

Some years after the establishment of these lines was begun, a third line was established by breeding and selection for both high milk yield and high fat test. This line is for the apparent purpose of serving as a control to compare with the crosses that will be made from the high milk yield, high test lines. This appeared to be about as good a breeding research project as was seen on this trip.

ARTIFICIAL INSEMINATION

The study group visited a number of artificial breeding centers in various parts of the Soviet Union. They also observed research in this area at several research institutes. The use of artificial insemination as a tool for herd improvement has increased rapidly during the past few years. It was stated that in 1958, 5,500,000 cows were bred by this method and that an estimated 8,000,000 cows would be bred during 1959. This figure included 1,000,000 cows owned privately by collective farmers. It appears that the present rapid increase in the use of artificial breeding of cattle is following the pattern established earlier with sheep. Over 30,000,000 ewes were bred artificially in the Soviet Union in 1958. Methods are under study and development for use with other species.

In most respects the artificial breeding program of the Soviet Union is similar to that of other countries. Stud farms are usually operated as part of a state farm under supervision of institutes or the Ministry of Agriculture. However, the artificial breeding center is usually budgeted separately from other farm operations. A typical center near Kiev had 50 bulls and 15 rams. In 1958, these sires provided semen for services to 52,330 cows in 200 cooperating collective and state farms in the area. Semen is priced on a per cubic centimeter basis that varies according to the quality of the sires and the volume of business for each cooperative farm. It was interesting to observe that the per-cow cost of breeding in the Soviet Union was comparable or higher than similar costs in the United States.

Techniques employed in artificial insemination were similar to ours, with a few exceptions. Fresh semen diluted with yolk citrate at rates of 1:20 to 1:40 is generally used throughout the country. The usual antibiotics are added and storage temperatures are similar to those used in most countries. All types of transportation, from bicycles to aircraft, are utilized. Shipments are made from the stud farms every three days.

Each collective or state farm employs a veterinarian and one or more lay technicians specially trained in artificial insemination techniques. Cows are inseminated as soon as heat is discovered and are rebred 10 hr. later if still in heat. The group was told that this universal practice of double breeding materially improved the conception rate. The large herds and availability of trained personnel make it feasible to employ this method. All inseminations are into the cervix and the speculum method is used at all times. Most other controls utilize the rectal palpation technique of insemination. However, the group was told on several occasions that the Soviet technicians had found the speculum method superior for their purposes.

The present use of artificial breeding and emphasis given to fundamental research in this area indicate that this method of breeding will play an important part in the future improvement of livestock in the Soviet Union.

SUMMARY

It is evident that since the end of the war and during the post-war reconstruction period Russian livestock specialists and farmers have made considerable progress in increasing the number of cattle kept for milk, the production per cow, and total output. At the same time, they have greatly improved the feeding and management conditions under which the cows are kept. There is every indication that this progress will continue. The Russians place great store in developing animals particularly adapted to specific environmental or regional conditions. They do not hesitate to mix breeds to attain this end. They attach little importance to frills, rather, they concentrate on utility items that will move them fastest to their goal of outdoing the United States in meat and milk production.

ASSOCIATION AFFAIRS

THE SOUTHERN DIVISION OF THE AMERICAN DAIRY SCIENCE ASSOCIATION HONORS

BEN EDMUND GOODALE

For Distinguished Service to the Dairy Industry in the South

Jackson, Mississippi — February 7, 1961

B. E. GOODALE was born in Marshalltown, Iowa, April 22, 1897, and received his pre-college education there. During his World War I service from 1917 to 1919 he was active in four major offensives; from France he received the Croix de Guerre, and from the U. S. Army the Silver Star Citation.

Professor Goodale received the B.S. and M.S. Degrees at Iowa State University, the latter in 1929, a double major in Dairy Manufacturing and Dairy Production. While he was still a student under the direction of the eminent Dr. Martin Mortensen, he operated the Iowa State (College) Creamery. He joined the Clemson College staff in 1922 as Associate Professor of Dairying, was advanced in 1944 to Professor, and in 1957 was named Head of the Dairy Department, which position he now holds.

Professor Goodale chose early to be a teacher, and in that position he has excelled, not only in instructing over 5,000 students on the Clemson campus but in carrying his knowledge as an Extension worker to Dairy Industry plants throughout the South and Midwest. He is especially known for his ability to counsel students on their academic affairs, and on social and religious matters as well. His service to Clemson College as Assistant Football Coach and Scout from 1922 to 1931, further attests to his intense interest in developing the whole student.

Fellow faculty members and the Clemson College community have recognized and benefited from the total pedagogical qualities of "Big Ben." He has served the Presbyterian Church as Deacon and as Lay Reader and has been instrumental in fostering interfaith understanding as Chairman of Clemson's Religious Emphasis programs. He founded the Clemson Health Clinic, has served as an advisor to the Y.M.C.A., and was appointed by the Governor of South Carolina to head up Civil Defense in Pickens County.

Professor Goodale is a member of the honorary fraternities Phi Eta Sigma, Alpha Zeta, Mu Beta Psi, Blue Key, and is a Fellow in Tau Kappa Epsilon. He is a member of the American Association for the Advancement of Science, American Dairy Science Association, and the American Association of University Professors. He is listed in American Men of Science, Who's Who in American Education, and Who's Who in the South and Southwest.

For 39 yr. Ben Goodale's students and friends have included him when they sing, "Where the Blue Ridge yawns its greatness . . ."

BOOK REVIEWS

Every Laboratory Needs the Ninth Edition of AOAC Official Methods

The ninth edition of the AOAC OFFICIAL METHODS OF ANALYSIS includes all changes adopted up to and including the annual meeting in October, 1959. The organization and presentation of tests are similar to earlier editions. Maximum use has been made of abbreviations, omitted prepositions, and cross references consistent with clarity. Even so, it has been necessary to increase the size of page and arrange the material in two columns per page in order to include the new material in a single volume. The Editorial Board and others who have contributed deserve much more credit than can be given in a review, for the monumental task they have completed.

Many new tests and changes in approved methods have been made necessary by the Pesticide Amendment of the Federal Food, Drug and Cosmetic Act. The rapid increase in new tests is shown by the great number of First Action tests which have been included. Approximately a fifth of the references cited in the 35-page chapter on Pesticides, and a fourth of the references on the 51-page chapter on Residues in Foods, were published after the eighth edition of Official Methods was published. Not only have new tests been developed and approved to answer new needs, but many modifications of older tests have been possible because of new laboratory equipment and techniques. Outstanding in the use of new techniques has been the adoption of chromatographic methods for a wide variety of tests. Every laboratory should have a copy of the Ninth Edition.

H. K. WILSON
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Urbana

NUTRITIONAL EVALUATION OF FOOD PROCESSING. Edited by R. S. Harris and H. von Loesecke. John Wiley & Sons, Inc., New York, N. Y. 612 pp. $12. 1960.

As stated in the preface, "If food processing is defined to include all treatments of a foodstuff from the place of origin to the point of consumption, then more than 95% of our food is processed." Here we have emphasized both the wide range of processing treatments considered and the importance of this subject to all concerned with the nation's food supply.

The introduction gives an excellent review of the stability of vitamins and essential amino acids to such processing factors and treatments as acidity, oxygen, heat, and light. Chapters of a general nature such as the effect of agricultural variables and the effect of commercial storage on the nutrient content are divided as to foods of plant origin and those of animal origin. The major food groups such as fruits and vegetables; meat, poultry, and fish; and oilseeds and oils are considered in separate chapters. Loss of nutrients during preparation for both institutional and home feeding is discussed in two chapters. Enrichment and standardization of processed foods with vitamins, minerals, and amino acids is also included.

Of special interest to readers of this JOURNAL will be the chapter entitled Effects of Commercial Processing of Milk and Milk Products on Nutrient Content by R. L. Wanner of Cornell University. He discusses nutrient effects of pasteurization, evaporation, condensation, and dehydration on milk, as well as processing of butter and cheese.

There are over 30 additional authors, each writing in a field of specialization. Their contributions vary from a few pages to a whole chapter. They have annotated the text with an extensive bibliography, so that the reader can find the source of almost every point made. The second chapter has over 300 references. Although there are no tables or graphs in the chapter on dairy products, most of the book is more than adequate in this respect.

There may be present some extraneous material, such as the composition of various packaging materials, and some repetition. In general, the book is well done and will be useful to all interested in the effect of food processing on nutritional content of foods.

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A BIBLIOGRAPHY OF FARM BUILDINGS RESEARCH, 1945-1958. Part IV: Buildings for Cattle. Agricultural Research Council, Cunard Building, 15 Regent Street, London, S.W.1. 1960.

The Agricultural Research Council has compiled a general bibliography of research publications on farm buildings. This is the first bibliography of this subject to be undertaken.

Buildings for Cattle is the fourth section of this bibliography to appear. It will be followed by other sections dealing with buildings for the processing and storage of grain and for the processing and storage of forage. There will also be a general section which will deal with miscellaneous types of buildings and with the construction and maintenance of farm buildings.

The bibliography considers farm buildings as part of farm equipment and gives references to design, construction, economics, and use. It includes the findings of scientific research, of such systematic but less precise forms of investigation as surveys, and of field trials and similar developmental phases of new techniques. It includes information from any country where

conditions are relevant to Great Britain. It gives abstracts of the original material and a review summarizing the main conclusions.

This bibliography covers the period from January 1, 1945, to April 30, 1958. The Council intends to keep it up-to-date by publishing supplementary bibliographies.

Buildings for Cattle (price 4/6d, by post 4/11d) can be obtained from the Agricultural Research Council, or from The A.R.C. Farm Buildings Unit, Wrest Park Lodge, Silsoe, Bedfordshire, England.

E. O. Herreid
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The Analysis of Fats and Oils. V. C. Mehlenbacher. The Garrard Press, Champaign, Illinois. 616 pp. 1960. $12.

As far as the reviewer is aware, this book is the most complete treatise available to date on the subject of methodology in the analysis of fats and oils. It brings up to date the subject material on methods in the American Chemical Society monograph series No. 58 Vegetable Fats and Oils by G. S. Jamieson and monograph series No. 41 Fundamentals of Dairy Science by the associates of Lore A. Rogers.

The Analysis of Fats and Oils is divided into eight chapters: Introduction, Fat Content, Estimation of Impurities, Stability, Specific Tests and Identification, Chemical Characteristics, Physical Characteristics, and Composition. The methods described in these chapters involve much of the same subject material covered in the Official and Tentative Methods of the American Oil Chemists' Society, as published by the American Oil Chemists' Society. However, it includes in addition, other methods not officially endorsed by the A.O.C.S. For example, the International Fat Commission method and the Wheeler method, as well as the official method of the A.O.C.S., are given for the determination of peroxides. The book thus allows for a choice of methods which best suits the analyst's need.

The discussion of dilatometry under the chapter on Physical Characteristics seems of special value to the dairy chemist. Dilatometric methods are seldom used to study the physical characteristics of butterfat by dairy chemists. The use of this method would be of value in a study of means to improve the spreadability of butterfat. Dr. Mehlenbacher discusses the use of gas chromatography in lipid analysis under the chapter headed Composition. Although the discussion of gas chromatography is somewhat brief, it indicates the comprehensive nature of the book. In fact, this book would serve as an excellent source for methodology in the entire field of lipids; its comprehensive nature serves to underscore the rapid rate of progress in the field of lipid chemistry.

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